Development of a method to clinically identify the position of the lingual nerve relative to the third molar region.

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By

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Abstract:

Introduction: Third molar extraction is a common surgical procedure in the oral cavity. During this procedure, the lingual nerve can be at risk of damage. This is due to its variable position in the lingual tissue and the lack of understanding of its *in-situ* position. The exact location of lingual nerve is difficult to predict as the nerve is not protected by bony canals and therefore not visible on routine pre-operative radiographs. Patients who experience nerve damage may suffer from persistent pain, sensory disturbances, and consequently, a poorer quality of life. Although lingual nerve position can be identified by magnetic resonance studies, this is expensive, not routinely done pre-operatively and can be difficult to be considered as a feasible method. The development of a simple method of detecting the lingual nerve clinically could help in understanding the position of nerve and aid in modifying surgical extraction technique whenever the nerve is located in a vulnerable position.

Aims:

1. To review the relevant literature of lingual nerve anatomy and identify the gap in the literature regarding the *in-situ* examination of the lingual nerve
2. To develop a method that can potentially identify the height of lingual nerve within the lingual tissue of the lower third molar region.
3. To test the reliability of this method by performing intra-observer and inter-observer agreement.
4. To corroborate the experimental clinical results with positional data from high-resolution MRI scans.

Methodology: A cross-sectional descriptive study on healthy participants was performed to investigate the ability of a standard electrical pulp tester device (EPT) in identifying the location of the lingual nerve (n=100). Ethical Committee approval was gained from Research Ethics Sub-committee for Physical Intervention before the research commenced. This research was

conducted in three studies. The first study was considered at its first part as a proof of concept which was conducted on 20 participants. This was mainly to check the ability of EPT in nerve stimulation and mapping the lingual nerve. Then, the recruitment was extended to include a further 30 participants. Nerve mapping procedure was carried out on both the right and left side by the same operator in this study. The second study was conducted parallel to the first study to investigate inter and intra observer reliability in 10% of the original sample size, 50 participants. The third study was performed on 5 participants (10%) by corroborating clinical findings from the nerve mapping phase of the study with confirmation gained from positional data from the MRI scans.
The mapping technique using EPT was performed by identifying the nerve in three different areas Points A, B and C. This was done in relation to clinical and anatomical landmarks intra-orally. Point A refers to the retromolar pad area which lies at, or in front of, the pterygomandibular raphe attachment. Point C referred to the distolingual attached gingivae of the erupted lower second molar. Point B referred to the midpoint of the attached lingual gingivae of the third molar crown. In the case of complete eruption, this becomes easy to identify, whereas in full, or partial, impaction this point had to be estimated as the midway point between Point A and Point C.

Results: In the first study, 50 healthy participants were recruited (22 males and 28 females). A total of 96 sides were mapped in the study (47 right 49 left). 87 lingual nerves were identified. The clinical mapping procedure was reported symptom free by most of the participants, apart from minor adverse events that were experienced by one participant. This was described as temporary paraesthesia, with the subject reporting this as a mild tingling of the tongue during eating. This completely resolved within three days. Simple descriptive statistics were performed to analyse the clinical data of nerve mapping in the first and second phases of the study. The mean values of position of points A, B, and C were, 9.60mm, 10.77mm, and 12.34mm respectively. An intra-class correlation coefficient (ICC) test was tested in 10% of the original sample to measure inter- and intra-observer agreement. For intra-observer agreement ICC results showed Point A= 0.82, point B=0.95, and point C=0.95. Inter-observer reliability between 2 different examiners showed ICC for point A= 0.96, point B=0.80 and point C= 0.93. These results considered to be good to

excellent agreement Test-retest agreement comparing the MRI and the EPT in 10% of the original sample using ICC showed point A with excellent agreement= 0.92, point B with good agreement= 0.82, and point C with moderate agreement= 0.68. The P value for the results in both the second and the third study was less than 0.003.

Conclusion: Within the limitations of this research, the aims and objectives were achieved. EPT was demonstrated an effective method for identifying the lingual nerve clinically and was associated with a very low rate of adverse events. This technique also showed good reliability between both intra- and inter-observer agreements. Comparison of both MRI and EPT have also shown a good agreement between the two methods in identifying a lingual nerve which also gives a further beneficial use for EPT apparatus.

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List of Abbreviations

EPT	Electric Pulp Testing
NICE	The National Institute for Health and Care Excellence
MARIARC	Magnetic Resonance Imaging and Analysis Research Centre
IAN	Inferior alveolar nerve
CBCT	Cone beam computerised tomography
Point A	Most posterior Point (represents the reference point of the retromolar pad)
Point B	Middle point (represents a reference point at the middle of the attached gingiva of the erupted third molar n case of full eruption and an imaginary point on the soft tissue where the crown of the third molar is likely to be.
Point C	Most anterior point (represents a reference point at the attached gingiva
MRI	Magnetic Resonance Imaging
NHS	National health services
HR-MRI	High Resolution Magnetic Resonance Imaging
SESPI	Research Ethics Subcommittee for Physical Interventions
LUDH	University of Liverpool Dental Hospital
PIL	Participant Information Leaflet
VAS	Visual Analog Scale
mm	Millimetres
3T	Three Tesla
7T	Seven Tesla
3D-DESS WE	3 Dimensional Double-Echo Steady with water excitation sequence
STIR	Short T1 inversion recovery
TR	Repetition time
TE	Echo Time
FOV	Field Of View
NEX	Number of excitation.

Chapter 1: Introduction

1.1 Overview of the problem

In any surgical procedure, safety measures should be considered to prevent unnecessary damage to vital structures. Although the healing capacity of the oral mucosa is considered to be high, some underlying vital structures, for example nerves, are vulnerable to long-term or even permanent damage. Lower third molar extraction is considered one of the most commonly performed surgical procedures in the oral cavity (Cheung *et al*, 2010). It is however associated with an incidence of nerve damage affecting either the inferior alveolar nerve or the lingual nerve (Cheung et al, 2010; Renton & Yilmaz, 2011). Several factors are related to a higher risk of nerve damage such as proximity of the nerve to the roots of wisdom teeth as well as other patient-related factors (Brann *et al*, 1999; Erdogmus *et al*, 2008). Some of these factors can be either studied clinically or by radiographic examination. Unlike the inferior alveolar nerve, the exact position of the lingual nerve, as it lies close to the lingual cortical plate, is considered unknown by most clinicians due to the inability to identify it in a routine pre-operative exam.

Lingual nerve damage has been reported to have a negative impact on the quality of life of most of the injured patients due to the impairment of: speech, swallowing, taste, and to a certain extent, persistent pain that can interfere with the normal activities of daily living. This has also raised medicolegal concerns that puts both the patient and the dentist in a stressful situation. The profession has therefore paid particular attention towards the importance of a pre-operative assessment of this nerve, as well as other nerves in the oral cavity, to reduce the risk of damage of the lingual nerve damage as a result of the nearby surgical procedure (Cespedes-Sanchez *et al*, 2014).

Electrical stimulation of nerve tissues has been used previously, either to test the effectiveness of anesthesia, or to identify the course of a nerve within the tissue prior to surgical procedures. Electrical stimulation of the lingual nerve was tested recently, in specific cases, as part of rehabilitation of salivary gland tissue and was found to be safe with no adverse side effects.(Wolff *et al*, 2018).

In this study, the aim was to assess whether an electric pulp testing apparatus could be used to determine the lingual nerve position.

1.2 Aims and objectives:

Aims

To devise a simple method of clinical assessment of nerve height within the lingual tissues using a readily available piece of dental equipment an electrical pulp tester (EPT). Developing a method that can potentially identify the risk of the lingual nerve damage, prior to mandibular third molar removal would have a direct impact on identifying surgical techniques which contribute to this damage. The results of this could help to avoid further surgical interventions required for nerve repair and recovery.

Objectives:

1. Review the literature of the available techniques in identifying the position of the lingual nerve and highlight the risk of its damage.
2. To evaluate the reliability of this new technique and identify its practicality and acceptance amongst the study population.
3. To assess the *in-situ* position of the lingual nerve in this study population and compare it with the anatomic location of the nerve that was reported in the literature.
4. To develop a protocol to view the lingual nerve on the Magnetic Resonance Imaging (MRI) scanning.
5. To correlate clinical findings of the lingual nerve position from this method with confirmed positional data gained from the MRI scanning.

Chapter 2: Literature review

2.1 Introduction:

Terminal branches of the mandibular nerve such as the buccal nerve, the inferior alveolar nerve and the lingual nerve, are at risk of damage following third molar extraction (Alling, 1986; Carmichael & McGowan, 1992; Pichler & Beirne, 2001; Sarikov & Juodzbalys, 2014). Implant placement, as well as local anesthetic injection around that area, have also been reported to contribute to nerve damage, although to a lesser extent compared to third molar extraction surgery (Berberi *et al*, 1993; Pogrel *et al*, 2003; Renton & McGurk, 2001). Amongst these branches of the mandibular nerve, the lingual nerve is considered at a great risk of damage, due to its close proximity to the operation site of mandibular 3rd molar (Bataineh, 2001; Blackburn & Bramley, 1989; Boffano *et al*, 2012; Fielding *et al*, 1997a; Mason, 1988; McGurk & Haskell, 1999; Mendes *et al*, 2014; Rood, 1992; Valmaseda-Castellón *et al*, 2000).

2.2 Incidence of the lingual nerve damage:

The true incidence of lingual nerve damage due to third molar removal can be difficult to predict because of multiple treatment modalities and variable contributing factors (Hillerup, 2007). The wide range of this reported incidence may also be explained by the noticeable heterogeneity of study designs and their inclusion criteria, different selected populations, the time of the assessment of nerve damage symptoms in relation to the surgical procedure, and the surgical technique undertaken around the third molar region (Loescher *et al*, 2003).

2.2.1 Incidence of the lingual nerve damage following mandibular third molar extraction:

A previous review of the literature has categorized the incidence according to the nature of the damage and the technique of extraction (Boffano et al, 2012). Following mandibular third molar extraction, temporary lingual nerve damage ranges between 0.6% (Goldberg *et al*, 1985) to 23% (Absi & Shepherd, 1993). Conversely, permanent nerve damage following third molar extraction is found to be less frequent and ranges between 0.5% and 8% (Blackburn & Bramley, 1989; Boffano et al, 2012; Rood, 1992; Zuniga *et al*, 1997).

2.2.2 Incidence of the lingual nerve damage as a result of local anesthesia injection:

Permanent nerve damage following local anaesthesia is considered to be a less common complication yet has been found to exist due to variable lingual nerve position. A simulated IAN block injection on 44 fixed cadavers has established that 4.5% (2 out of 44) injections will have penetrated the lingual nerve (Morris *et al*, 2010). In a clinical situation, Krafft & Hikel (1994) have investigated the sensory disturbance of the lingual nerve following mandibular block anaesthesia administration in 12104 patients. Those patients did not have a surgical procedure to eliminate introducing a confounding factor to the results. These authors observed that 0.15% (n=18) of patients reported a temporary sensory disturbance. Only one patient (0.008%) had a permanent sensory disturbance.

The lingual nerve gets affected relatively more when mandibular block anaesthesia is administered compared to the inferior alveolar nerve by 70% vs 30% respectively (Pogrel et al, 2003). Dissecting the lingual and inferior alveolar nerves in a cadaver studies have demonstrated that the uni-fascicular structure of the lingual nerve compared to the poly-fascicular nature of the inferior alveolar nerve can be a potential contributor to this phenomenon (Pogrel et al, 2003; Tan *et al*, 2014).

2.3 Types of the lingual nerve injury:

Nerve injury can occur as a result of direct or indirect trauma. The proximity of the lingual nerve to the surgical operation site can make it vulnerable to different types of injuries (Pogrel *et al*, 1995; Yadav *et al*, 2014).

'Stretch' trauma can happen when the forces applied to the nerve is more than the elastic capacity of the nerve body (Burnett & Zager, 2004). This can happen during third molar extraction surgery, especially upon retraction of the lingual flap which is intended to protect the exposed lingual nerve from damage. Insufficient lingual flap exposure was also found to contribute to this type of trauma. This was explained by the lack of understanding of the exact point of retraction and inadequate protection of the lingual side of the flap (Hupp, 2007).

Laceration injuries can happen to the lingual nerve as a result of a sharp instrument which cuts the nerve partially or completely (Boffano et al, 2012). This can happen initially upon incision, or during flap reflection, or following socket curettage using sharp instruments like a bone chisel or burs (Burnett & Zager, 2004; Hupp, 2007).

Compression injury is the least common injury to the lingual nerve and can happen to the nerve as a result of ischemia to the nerve without damaging its integrity (Burnett & Zager, 2004).

Whilst physical trauma is the most common risk, other types of injury can occur to the lingual nerve as a result of different procedures that are carried out in that area. Thermal damage can occur as a result of insufficient coolant of the hand-piece whenever a rotary instrument is required for bone removal (Hupp, 2007). Chemical damage to the nerve can also result to the nerve whenever it is in direct contact with a chemical material (Hupp, 2007).

2.4 Overview of nerve injury classification in the literature:

Nerve injury classifications such as Sunderland and Seddon have been used by clinicians to aid in injury grading, and its treatment (Chhabra *et al*, 2014). In 1943, Seddon described the three main presentations of nerve injury as:

Neurapraxia: first-degree nerve injury that leads to temporary block of the conduction and demyelination.

Axonotmesis: second-degree nerve injury which results from loss of axons while the connective tissue is preserved.

Neurotmesis: third-degree nerve injury which is its most severe form where the nerve is severed physically (Seddon, 1943).

This simple classification is easily understandable; however, a more detailed classification, that is able to distinguish between different nerve tissue involvement, is important, especially when nerve repair is considered (Chhabra et al, 2014).

Sunderland (1951) expanded nerve injury categories into 5 degrees. The first and the second degrees are described in the same way proposed by Seddon (Seddon, 1943). However, the third-degree injury in Sunderland's classification describes the injury to endoneurial tubes; while the fourth-degree describes the injury to the perineurium. The final, fifth, degree describes the injury to the epineurium which was considered to be the most severe type of nerve injury (Sunderland, 1951).

A further modification to the Sunderland classification was performed by adding a sixth degree of nerve trauma, which involves various layers of the nerve rather than only one (Mackinnon & Dellon, 1988). This was proposed to recognise that most cases of nerve injury don't occur typically according to the classical model of nerve injury as a mix of variable tissues is usually involved in the trauma.

2.5 Signs and symptoms of patients with an injured lingual nerve:

The damage of the lingual nerve may lead to disordered sensations in the corresponding innervated tissues (Berberi et al, 1993; Hillerup & Stoltze, 2007a; Hillerup & Stoltze, 2007b). Symptoms, most of which are subjectively reported by patients experiencing nerve damage, include numbness, pain, tingling, and altered taste (Zuniga & Essick, 1992). Signs of sensory disturbances may be described by the clinician into different categories such as hypoaesthesia, which is reduced sensation, or paraesthesia, which is an abnormal sensation (Fielding *et al*, 1997b) or, in some cases, as dysaesthesia which is unpleasant abnormal sensation (Boffano et al, 2012; Loescher et al, 2003; Mason, 1988).

Moreover, pain modulation with altered sensations is found to be associated with the damage of lingual nerve (Hillerup & Stoltze, 2007b). For example, allodynia, which is the experience of pain from non-painful stimuli, or the development of an exaggerated pain response to painful stimuli (hyperalgesia), are frequently observed in this group of patients (Hillerup & Stoltze, 2007b; Jørum *et al*, 2003).,

2.6 Quality of life associated with patient with an injured lingual nerve:

Patients with persistent lingual nerve damage after surgery have a significantly poorer health-related quality of life as well as poorer oral health-related quality of life than those who do not suffer from this surgical complication.

Sensory disturbances can affect the patient's ability to perform normal functions such as chewing, speaking and food maintenance (Boffano et al, 2012) but some effects can also have life-changing consequences. For example, an impaired taste sensation can have deleterious effects in professions such as catering, cooking and professional tasters. Difficulty in speaking can have a significant impact on the work lives of those who need to use their voice for communication, e.g. lecturers, actors, salespersons and, especially, for professional speakers (Blackburn, 1990).

The experience of persistent pain sensation over the branches of the injured nerve can also have negative effects on the social life of some individuals. Those patients who reported a decrease in life satisfaction are more likely to suffer from depressive symptoms (Leung *et al*, 2013). An available literature has shown that the lingual nerve damage remains one of the reasons involved in complaints and litigation as a result of psychological distress and discomfort (Ferrús-Torres *et al*, 2011; Loescher et al, 2003).

2.7 Factors affecting the lingual nerve injury:

The factors which influence the lingual nerve damage have been studied in the literature. Surgical technique in lower third molar extraction appears to have a great influence (Rood, 1992). Although raising a lingual flap has been recommended to facilitate access and visibility to the extraction site (Pogrel & Goldman, 2004), reviewing the literature has uncovered a strong correlation between temporary lingual nerve damage and lingual flap reflection (Bataineh, 2001;

Mason, 1988; Pichler & Beirne, 2001; Robinson & Smith, 1996). Other factors have influenced lingual nerve injury during extraction such distal bone removal (Blackburn & Bramley, 1989; Rood, 1992) and lingual retractor size (Greenwood *et al*, 1994). The use of different elevators to elevate the lingual mucoperiosteum has also been found to contribute to temporary nerve damage. This was found with a variable incidence ranging between 6.6% (Rood, 1992) and 22% (Fielding et al, 1997a).

Performing lower third molar extraction under general anaesthesia has also been found to increase lingual nerve damage five times more frequently than surgical procedures performed under local anesthesia (Brann et al, 1999). It was suggested that this was possibly due to the supine position of the patient and the relatively higher degree of surgical difficulty in most of the general anaesthetic cases. Partial eruption of lower wisdom and the age of the patient (Valmaseda-Castellón et al, 2000), as well as the inexperience of the operator have also been found to increase the prevalence of the lingual nerve damage (Bataineh, 2001; Jerjes *et al*, 2006).

The position of the third molar and its level of impaction can also be a risk factor for lingual nerve injury (Baqain *et al*, 2010). This was explained by horizontal, or mesio-angular, angulations of the tooth dictating a difficulty of the extraction technique which might require bone removal and extended operation time, that can result in iatrogenic damage to the lingual nerve (Baqain et al, 2010).

2.8 Pre-operative risk assessment:

In the case of the inferior alveolar nerve, assessment of the nerve position prior to third molar surgery is relatively straightforward. As the shape of the canal within which this nerve runs is readily visible on radiographic images and is a reliable predictor of inferior alveolar nerve injury during third molar surgery (Shiratori *et al*, 2013). This assessment is mostly undertaken from analysis of routine

orthopantographic or cone beam computed tomography images (Ueda *et al*, 2012). The body of work which has determined the risk factors and risk assessment of the inferior alveolar nerve have led to significant changes in clinical practice being adopted. It has been pivotal in the development of clinical guidelines, including The National Institute for Health and Care Excellence (NICE) recommendations, on third molar removal and informed the development of new surgical techniques.

Attempts to visualise the in-situ position of the lingual nerve, as reported in the literature, have not been easy (Cox *et al*, 2016; Fujii *et al*, 2015b; Kiesselbach & Chamberlain, 1984; Miloro *et al*, 1997). Magnetic Resonance Imaging (MRI) is considered one of the best methods to visualise the cranial nerves *in-situ*. The development of high-resolution magnetic resonance imaging and the advancement of imaging protocols has made the visualisation of terminal nerve branches possible. Although this technique is considered to be safe, from a patient-safety point of view as it is radiation-free, it is however, highly specialized, inconvenient and cannot be considered routinely, as the feasibility of using this technique pre-operatively is relatively impractical and expensive.

2.9 Anatomy of the lingual nerve:

The lingual nerve is a branch from the third division of the trigeminal nerve after it exits the foramen ovale. It provides sensory innervation to the anterior two thirds of the tongue (Drake *et al*, 2009). Anastomoses of the lingual nerve with different cranial nerves along its distribution can also happen on different levels with different nerve fibers (Erdogmus et al, 2008). Taste fibers from the Chorda tympani of the facial nerve (VII) join the lingual nerve about 15 mm following its exit from the foramen Ovale (Erdogmus et al, 2008) following its bifurcation from the inferior alveolar nerve (Sittitavornwong *et al*, 2017). Combined, these fibers provide afferent nerve fibers that carry thermal

and mechanical and gustatory signals from the anterior two-thirds of the tongue.

Along the body of the nerve, the lingual nerve can show variable cross-sectional morphology including oval, round and flat, and the size can vary between 1.53mm to 4.5mm (Hölzle & Wolff, 2001). The course of the lingual nerve in the infratemporal fossa was observed to be running lateral to the medial pterygoid, and inferior-medial to the Inferior Alveolar nerve (IAN) (Piagkou *et al*, 2011). The lingual nerve is sandwiched between the tensor veli palatini and the lateral pterygoid muscles in which it meets the Chorda tympani. Moving downwards, and in contact with the medial pterygoid muscle, the lingual nerve contacts the medial aspect of the mandibular ramus, where it then passes inferior to the superior constrictor and pterygomandibular raphe in close contact to the mucoperiosteal tissues of the lingual aspect of the mandible. The lingual nerve then continues moving forward, superior to the mylohyoid muscle, and then courses in proximity with the superior part of submandibular gland providing parasympathetic innervation to the gland via the submandibular ganglion (Drake et al, 2009; Piagkou et al, 2011).

Efforts have been made to define the location of the lingual nerve around the third molar area. This has been studied and investigated by cadaveric dissection and imaging studies (Behnia *et al*, 2000; Benninger *et al*, 2013; Chan *et al*, 2010; Erdogmus et al, 2008; Karakas *et al*, 2007; Kiesselbach & Chamberlain, 1984; Kim *et al*, 2004; Mendes et al, 2014; Miloro et al, 1997; Pogrel et al, 1995; shinora *et al*, 2010). The descriptions of the vertical height and horizontal distance in relation to the third molar lack consistency across these studies due to their different methods and sample sizes.

2.10 Methods of identifying position of the lingual nerve in the third molar region.

The available literature demonstrates that the course of the lingual nerve has been investigated extensively using different techniques. The most abundant data about the location and its relation to adjacent structures has been obtained directly via dissection studies (Behnia et al, 2000; Kiesselbach & Chamberlain, 1984; Kim et al, 2004; Pogrel et al, 1995). Due to the suggestion that there is less accuracy of results from dissection studies, as a result of tissue fixation and distortion, indirect observation of the lingual nerve has been reported using different imaging techniques such as MRI and Ultrasonography. (Miloro et al, 1997; Olsen *et al*, 2007).

2.10.1 Dissection studies combined with clinical assessment:

Kieselbach & Chamberlain (1984) have observed the lingual nerve using two different approaches. The first one involved 34 dissected cadavers that highlighted a considerable number of the lingual nerves (17.6 %) to be at, or above, the level of the alveolar crest. It was also observed that the horizontal relationship between the lingual nerve and the lingual plate was found to be as close as 1mm, which potentially can be the thickness of the periodontium. The second, clinical, approach observed the *in-situ* position of the lingual nerve following reflection of a lingual flap during third molar extraction surgery. Although this technique is no longer advocated, because of the risk of damaging the lingual nerve (Appiah-Anane & Appiah-Anane, 1997; Gargallo-Albiol *et al*, 2000; Robinson & Smith, 1996), Kieselbach & Chamberlain noted that only 4.6% of the lingual nerves (of their 256 cases) were superficially placed on the alveolar crest.

2.10.2 Cadaver studies with direct measurement of the lingual nerve:

Pogrel et al (1995) also studied the lingual nerve in the mandibular third molar region. In these cases, a 20 dissected cadavers were

edentulous and showed variable vertical and horizontal positioning of the lingual nerve. The lingual nerve in 3 specimens was identified to be at or above the lingual crest, which indicates the vulnerable position of the nerve. The mean horizontal distance of the lingual nerve to the lingual plate was found to be 3.45mm and the vertical distance was found to be 8.32mm. Pogrel et al (1995) also stated that the position of the nerve on right side had no direct correlation to the position of the nerve on the left side. In 2001, Hölzle & Wolff performed a similar study to that of Pogrel et al., with slight differences in the sample size. 68 dissected nerves, in 34 cadaver heads with atrophic mandibles, were analyzed. This latter study recorded that the distance of the lingual nerve from the lingual crest was reduced in atrophic mandibles. They also emphasized the findings by Pogrel et al. (1995), regarding no correspondence of the right and left horizontal and vertical measurements of the lingual nerve.

Mendes et al. (2013) dissected 24 half head cadaveric specimens to observe the course of the lingual nerve distribution around fully erupted third molars. The measurements were taken following the determination of certain points (the lingual nerve and the retromolar pad area, the lingual nerve and the alveolar rim of the third molar) and the distances between them measured. This group's findings stated that the average vertical position of the lingual nerve, in relation to the lingual alveolar rim of the corresponding third molar, was found to be around 16.8 mm. Compared to previous studies findings this investigation stated that the nerve can also be located deep in the lingual tissue. It also highlighted the close relationship of this nerve to the cortical plate, which can leave its integrity at risk whenever a surgical procedure is taking place (Mendes *et al*, 2013).

In a study with a larger sample size, there was investigation of the anatomy of the lingual nerve around mandibular third molars (Behnia et al, 2000). 669 lingual nerves were studied from fresh cadavers. The study noted that 14% of the dissected nerves were located above, or at, the lingual crest. In one specimen, only one nerve was found in the

retromolar pad area (0.1514%). The authors postulated that this unusual position of the nerve was due to the possibility that it had been displaced as a result of previous trauma to the face, causing this exceptional presentation (Behnia et al, 2000). These authors also investigated the horizontal relationship of the lingual nerves to the lingual plate in this study. They reported that 22% of the lingual nerves in these specimens were found to be in direct contact with the lingual plate. To reduce the risk of the lingual nerve displacement during the investigation, they used 2 clippers to stabilize the position of the nerve, after the process of dissection to 'fix' the nerves. The cadavers were also dissected within 24 hours of death which may have been a factor in the position of the dissected, exposed nerves, being close to that predicted by *in-situ* studies (as there would be less time for *post mortem* changes to have occurred). The relatively large sample size of this study also imply more reliable results compared to low sample size studies.

2.10.3 Imaging of the lingual nerve:

1. Radiographic technique of cadaver studies:

The distribution of the lingual nerve in the oral cavity is submucosal. No bony canal or hard tissue reference can therefore be used as a radiographic marker to identify the actual distribution of this nerve in the oral cavity. Hence, it is impossible to determine its location on radiographic imaging, compared to the inferior alveolar nerve or mental nerve which can be identified by locating the bony canal and the mental foramen (Greenstein & Tarnow, 2006; Liu *et al*, 2009).

In cadavers, it became possible to utilize radiographic imaging of dissected tissue to study the location and the distribution of trigeminal nerve branches, like the lingual, and inferior alveolar nerves, by introducing radiopaque material into the dissected samples. Those nerves were studied in the most dentally relevant area around the infratemporal fossa, and the medial aspect of the mandible before it

merges with the tongue. (Chan et al, 2010; Karakas et al, 2007; Kim et al, 2004). In a Korean study in 2004, 32 hemi-sectioned heads had their lingual nerve painted with water-soluble barium. This was followed by radiographic imaging of each cadaver to view the topography of the lingual nerve. After radiographic tracing, the lingual nerve appeared to be related to the retromolar pad area vertically, with an average distance of 7.8mm from this structure.

Chan et al. in 2010, studied the position of the lingual nerve in the molar and premolar region by dissecting 18 cadaver heads and comparing these to clinical results gained from Cone Beam Computerised Tomography (CBCT). Clinically, 75% of the sampled nerves were found to diverge towards the tongue at the first and second molar (Chan et al, 2010). The vertical distance from the cementoenamel junction of erupted teeth to the lingual nerve was 9.6mm, 13mm and 14mm at the second molar, first molar and second mandibular premolar respectively. The radiographed cadaveric sample, which was selected randomly, was prepared by inserting a metal wire in the exposed lingual nerve. The difference between the clinical and mean radiographic measurements were found to be minimal (0.57 +/- 2.62mm).

Another group of investigators studied the lingual nerve and its relation to the mandibular third molar by implementing radiographic imaging of cadavers. Karakas and his collegues have employed radiographic markers in 10 dissected cadavers (Karakas et al, 2007). The technique used involved adapting a metal wire to the exposed lingual nerve, to enable it to be viewed radiographically. The mean vertical distance between the lingual nerve and the alveolar crest was 9.5 ± 5.2 mm. The mean horizontal distance between the lingual nerve and the inner aspect of the lingual plate was 4.1 ± 1.9 mm. In this study, the group have also investigated the cross-section of the nerve and reported that the majority of the studied lingual nerves, 81%, were round and the rest were found to be flat. The small sample size of this study and the methods of wire adaptation may, however, provide inaccurate results

as radiographic measurements, in studies such as this, may suffer from magnification errors (Karakas et al, 2007).

2. Ultrasonography technique in clinical and cadaver studies:

An ultrasound scan is a technique that creates an image of the structure by using high-frequency sound waves. Benninger et al., in 2013 have utilized this imaging technique on both cadavers and clinical volunteers to investigate the feasibility of using ultrasonography to image the lingual nerve. They dissected 28 lingual nerves on cadavers and found that 79% of the nerves were sitting on the crest of the lingual plate. In the clinical part of the study, 70 volunteers had their lingual nerve scanned and identified, on both sides, with the ultrasonography apparatus. Although no specific measurements were taken clinically to locate the exact position of the lingual nerve, this technique enabled the authors to classify the height of the nerve in that area, based on 'high' or 'low' criteria. Another study investigated ultrasonography on animal cadavers and confirmed the feasibility of this technique in identifying the lingual nerve (Olsen et al, 2007). Although these investigations have concluded the usefulness of using ultrasound to identify the lingual nerve, they have been limited by small sample size and unclear detail as to the methodology used, which makes them difficult to reproduce. It is important to add that the availability of the ultrasonography machine amongst dental practitioners, as well as the need for a prerequisite training, have posed significant drawbacks in making this a routinely adopted technique.

3. Magnetic resonance studies (MRI):

The first clinical study of the lingual nerve position using this imaging technique was done my Miloro et al. in 1997. They scanned 10 healthy subjects with high-resolution MRI (HR-MRI) and adopted a specific protocol in order to view the nerve in the third molar region (Miloro et al, 1997). In this study, Miloro's group were able to investigate the horizontal and the vertical relation of the nerve to the lingual crest and

plate. They found that the nerve can be superior to the alveolar crest in 10% of cases but 25% were in direct contact with the lingual plate.

MRI techniques have improved recently in the last decade. By utilizing a different MRI scanning protocol of the previous study, Fuji and colleagues were able to visualise the branches of trigeminal nerves and found that lingual and inferior alveolar nerve can be detected (Fujii et al, 2015b). Importantly, however, these nerve branches were studied before the nerve entered the oral cavity and merged with the complex structures there. MRI has also been used to detect suspected peripheral nerve injuries, which can help aiding a proper diagnosis and identify the source of the problem (Cox et al, 2016).

2.11 Conclusion:

Pre-operative risk assessment of the lingual nerve by identifying its *in-situ* position around the third molar region is not routinely performed, largely due to the need for more sophisticated imaging techniques such as high-resolution MRI scanning to visualise it which are not considered available for that purpose. Instead, it is recommended that the raising of lingual tissue be avoided, and care is taken not to perforate the lingual bone, during drill use. While the nerve is at risk during these two scenarios, it is also at risk of damage when using sharp instruments in its vicinity and during suturing - especially when it is located high in the lingual tissues, at or above the level of the lingual alveolar crest. Preoperative knowledge of the lingual nerve position would alert the clinician to the potential of encountering it in these scenarios. In this research, a readily available tool within the dental surgery, EPT, was investigated to identify whether it is possible to be utilised in a routine pre-operative assessment of the lingual nerve position.

Chapter 3: Development of Nerve mapping technique: cross sectional observational and feasibility study:

3.1 Introduction

Nerve mapping is a technique that uses an electrical current from an electrode tip whereby the superficial branches of the nerve can be identified and located either submucosally or subcutaneously. Preoperative nerve mapping has been shown to be useful in the head and neck region, especially before conducting surgical procedures near the facial nerve (Bo *et al*, 2015; Park, 1998). The mapping of the facial nerve involves observing its motor activity to the muscles of the face. This can be done by testing whether the nerve is blocked or not by detecting any active muscle movement whenever the nerve is tested.

3.1.1 Physiology of electric stimulation of nerve fibers:

When an electrode (such as that found in an electric pulp tester tip) is placed on the soft tissue, the electric stimulus induces ionic changes of the membrane of the nerve thereby creating an action potential that will be transmitted along the nerve body (Bender, 2000). Distinct nerve fibre classes differ in their degree of myelination and their diameter. These features can determine the electrophysiological properties and the speed of action potential propagation. To induce an action potential the stimulus must reduce the membrane potential to its threshold of activation. This requires a change in voltage across the membrane and activation of voltage gated ion channels. The response of the membrane potential to a stimulus is dependent on the distribution of ion channels along the membrane surface and therefore its capacitance. For instance, Aβ fibres are myelinated with a high density of ion channels collected at the nodes of Ranvier. This concentration of ion channels has a low capacitance and thus a short duration of voltage stimulus is enough to change the membrane potential to threshold. Whereas C fibres are unmyelinated and have ion channels distributed more diffusely over the entire surface of the membrane

resulting in a larger capacitance and therefore requiring a longer duration of stimulus to move the membrane potential to threshold (Sippel & van Zundert, 2012).The device used in this study has a short pulse duration which selectively activates large myelinated sensory nerve fibers. This reduces the chances of stimulating C nerve fibers which can create painful or unpleasant sensation.

Individuals who experience nerve activation using an electrical stimulus are expected to interprete their reaction as either specific sensation such as tingling, burning, pinprick or muscle twitch depending on the nerve type (motor or sensory). In the case of lingual nerve activation, placing the electrode tip distant to the nerve produces a mild tingling sensation in the immediate location of the electrode tip. However, when near the nerve, it produces a recognisable sensation projected tingling sensation along the distribution of the nerve which represets the epsilateral side of the tongue.

3.1.2 Overview of electric pulp testing:

Electric pulp testing (EPT) has been a standard method used in dentistry over many decades to test if the nerves within the tooth pulp are able to conduct sensation or have become non-vital secondary to infection or trauma. The process involves passing a small electrical impulse of a fixed direct current through the dental tissues and, if the pulpal nerves are intact, will elicit a mild sensation in the subject. Due to the design of the device, the duration of this stimulus is controlled by the patient, if he/she wishes to stop the stimulus they need only to release contact with the device, thus breaking the electrical circuit and halting the stimulus.

Use of EPT was first reported by Bjorn in 1947 and Harris in 1956, (Lin & Chandler, 2008). Agren & Danielsson in 1980 used the electric pulp tester to evaluate the depth of local anaesthesia gained in clinical studies, comparing various methods of local anaesthetic application. EPT has also been shown to be successful when testing the effectiveness of local anaesthesia on the lower lip after administration

of an inferior dental block (IDB) in a group of healthy volunteers (Ku *et al*, 2011). Although designed to test pulp sensibility, EPT has proven to be a reproducible method to assess soft tissue sensation (Dal Santo *et al*, 1992). It has also been compared with various methods in detecting mucogingival sensitivity (Walline *et al*, 2000). All this data from the literature suggests that the use of EPT could be used as a safe tool on the soft tissue with no incidence of tissue damage (McDaniel *et al*, 1973).

Study Aims:

This study aimed to test the use of electric pulp testing on healthy participants to identify:

1. Its ability to stimulate the lingual nerve tissue located submucosal and lingual to the third molar.
2. To map the lingual nerve clinically and get positional data of the lingual nerve from both right and left side of the recruited sample and compare it with the reviewed literature.

3.2 Methodology:

3.2.1 Study design:

The present investigation was designed as a cross-sectional descriptive feasibility study, mapping the lingual nerve as a chair-side experiment in healthy participants. The study had two parts. In the first part, participants were invited to attend a one-hour session, in which data collection of nerve stimulation and nerve mapping of both right and left lingual nerves were conducted. The second part was an extension of recruitment and a continuation of the same nerve mapping procedure of the first phase.

3.2.2 Application for ethical approval:

The application form for approval of a project involving human participation, human data, or human material was completed and sent to the Ethics Committee at the University of Liverpool with attached documents on the 23rd of February 2016. That Committee suggested submitting the ethics application to the Research Ethics Subcommittee for Physical Interventions (SESPI) as the study was considered to involve more than minimal risk to the participants. Both the research supervisor and the student investigator attended the Committee meeting for ethical application review, held in the Foundation Building, University of Liverpool on the 21st of April 2016. The feedback on both the application and the attached documents were taken into consideration. Further documents were added to the amended documents, such as patient information leaflets for the MRI part of the study and a completed risk assessment form, before the resubmission for the final review. Ethics was gained via an approval letter which included further details and conditions of the approval. The application, risk assessment form and ethics Approval Letter are attached (Appendices 1-3).

Ethical considerations:

Participants should be safeguarded and protected from any physical or psychological harm when they take part in an experimental study. Their private information, as well as their needs, should be protected. Since this was a healthy subject recruitment, university students were invited to participate. Recruitment was completely voluntary; no pressure was exerted on the students to commit to the study. Likewise, if they did join the study, there were no benefits conferred, and their participation would not affect their progress.

There were cases where a participant wished to consider undergoing the removal of a wisdom tooth subsequent to the study. In this instance information gained during the study i.e. identification of a high-risk lingual nerve position, might be of benefit to the planning of the surgical procedure and therefore this information was provided to the subject for communication to their surgeon.

Participants were informed of the experimental protocol before the start of the procedure. Foremost they were informed that the procedure had the potential to produce a mildly uncomfortable sensation, if the nerve was stimulated at a higher setting. They were made aware that this procedure was fully under their control and shown how to stop the stimulation to minimize this discomfort if it occurred. They were also informed that consent could be withdrawn at any point, without the need for explanation.

It was ensured that recruited subjects were able to differentiate between a sensation confined to the area around the probe tip and that of a projected sensation on the tongue. They also had to be able to communicate this to the investigator by either vocalization or hand/other gesture. Efforts were also made to facilitate communication by a picture board, to allow participants to communicate without speech where needed. All participant information was translated where necessary into the subject's preferred language.

Reimbursement of participant expenses:

It was acknowledged that this study required attendance at one appointment and could have been considered as an inconvenience to the subjects. As a part of participation this study offered a small reimbursement of no more than £10 to compensate for this inconvenience. This amount of money was not considered as an incentive or bribery to the subjects to convince them to commit to the study as their participation was purely voluntary. It was given to the participants as a pre-paid voucher at the same day as their attendance.

3.2.3 Sample selection and recruitment :

- **Inclusion criteria**

The criteria on including participants in this study were made clear and simple to facilitate recruitment and fulfill the study objectives. Healthy adult subjects with no history of extraction of lower wisdom teeth and lower permanent molars, who were a minimum of 18 years of age with no oral or mucosal ulcerations present were considered eligible for recruitment. As the study was conducted at the Dental School, undergraduate students as well as staff members, clinicians and teachers were considered good candidates for the study. Hence as the aim of this study would be fulfilled while recruiting healthy participants, recruiting NHS patients would not confer any benefit in this study. In fact, it may have challenged the award of the original ethical approval as well as including many variables that could affect the distribution of the data.

➢ Exclusion Criteria:

The criteria to exclude potential the participants were as follow:
1. The presence of electrically implanted devices. These devices might be affected by the current generated by the electrical pulp testing impulses. To remove the risk of interference, any participant in this category was not recruited.

2. A known gag response that would cause subject distress and interfere with performing the nerve mapping procedure. In participants who has already reported a noticeable gag reflex, they were excluded from further participation. Any, incidental, mild gag reflex that was found during the procedure was considered as normal as the area of study was considered to be sensitive, and a mild gag reflex was expected.

3. Subjects without capacity or inability to consent. Gaining of valid consent was considered to be crucial to be able to take part in the study. Participants who had learning disability or were unable to understand the concept of the experiment were not asked for participation in this study.

4. Ulceration or similar condition of the gum in the area being studied. Healthy soft tissue with an absence of any ulceration, periodontal disease or any abnormal soft tissue condition was ensured. Participants with existing intra-oral ulceration or soft tissue disorders were not recruited in the study as the mapping of the lingual nerve might induce irritation and pain, especially on an already diseased or compromised soft tissue.

5. Known neuropathy or pain in the nerve being studied. The lingual nerve is a branch of the trigeminal nerve and therefore any participants with a history of, or known existing neuropathic pain in the trigeminal nerve or facial nerve were excluded, as the use of electric pulp testing might cause an adverse reaction.

6. Previous surgical procedure related to extracted/ decoronated mandibular third molar, or the knowledge of congenitally missing ones. The liklihood of performing a surgical procedure around the lingual nerve area is the extraction of the lower third molar. Participants who have had their lower third molar extracted or decoronated were excluded from the study as there may have been peri-operative trauma or post-operative changes

7. Unable to give consent.

8. Participants under the age of 18 years old.

9. Participants with the hisotry of oral cancer or radiotherapy in the head and neck.

Recruitment process:

Recruitment of subjects was undertaken in three different ways and was as follows:

1. **Posters**

Designated advertisement posters were attached to specific notice boards with high visibility positions on university property. Care was taken to ensure that only the approved poster was placed in appropriate sites. A copy of the poster is attached (Appendix 4).

2. **E-mail**

An email with a short text explaining the aims and methods of the study, and containing contact information details, was sent to key secretarial staff for distribution to staff or students of the university via their university email account. One follow-up email was sent as a reminder. A copy of the email content is attached in (Appendix 5).

3. **Oral presentation**

With the agreement of the lecturer, an oral presentation about the project was given to the audience; students or staff members to

verbally draw their attention to the study, after the lecture or meeting had finished. A 5-minute brief verbal presentation was delivered, on each occasion giving the audience contact details to discuss any further details twice. It was made clear that participation was not mandatory, and the audience was allowed to ask questions or inquire about any further details during the oral presentation.

Sample population and sample size justification:

From anatomical studies, it was estimated that branches of the trigeminal nerve (especially the lingual branch) may be in a surgically unfavourable position in approximately 10% of the population. To be reasonably certain that a similar proportion of subjects could be identified and accurately calculated in a random population, the statistician advised that a minimum of 50 participants should be recruited.in addition, the clinical tests were completed in a short timeframe and were not onerous on the part of the participant or researcher. Therefore, it was realistic to assume that this number of subjects could be recruited and tested within the timeframe given. Participants were recruited from the healthy adult population.

Following the full recruitment process, 55 participants voluntarily agreed to take part of the study. However, five of these didn't meet the inclusion criteria, either due to a severe gag reflex, a previous history of neuropathic pain in the head and neck region, or limited availability in the time allocated for the research project.

3.2.4 Venue:

The study was held in a mixed clinical/university room, number G02 in the University of Liverpool Dental Hospital (LUDH), which is designed for the purpose of recruiting healthy subjects (not NHS patients) in a clinical environment. This clinic is located on the ground floor of the Dental Hospital at the end of the Oral Surgery department. Subjects who agreed to participate in this experiment were asked to come to

Reception 3, and the student investigator (SA) met them there to help guide them to G02.

3.2.5 Nerve mapping procedure using EPT:

The EPT as any electric machine has a standard output property. The current in this machine is considered constant with a manually adjusted voltage (5-400 volts) to account for different tissue resistance. The waveform of this current was investigated using an Oscilloscope. This was performed to ensure avoiding activating C fibres and to primarily activate Aδ fibres upon soft tissue application. The following figures (3.1 A, B) show the typical output of the machine over a period of time and an expanded single pulse with its exact duration.

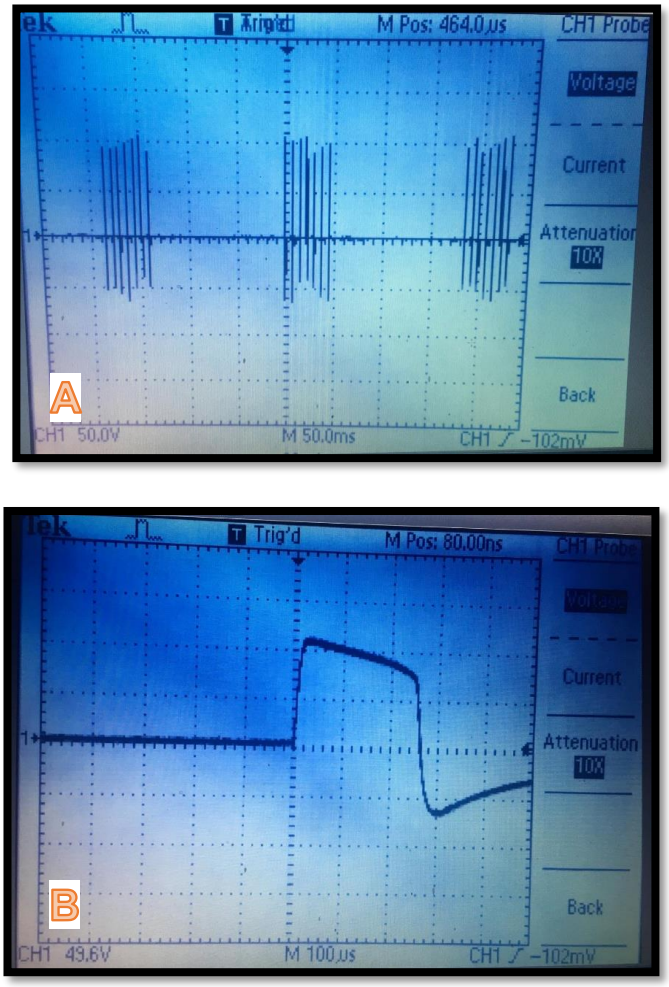

Figure 3. 1 Showing Oscilloscope analysis of EPT impulses over a tested period of time. A shows the typical output of seven pulses over a period of 50 milliseconds and intervals of 130ms between pulses. B shows an expanded single pulse with an approximate square wave waveform of 250 microsecond's duration.

Data on the nerve position were collected in both parts of this study, and together this data formed a cross-sectional study design. The availability of the equipment was guaranteed beforehand to prevent any inconvenient shortage throughout the procedure. The battery charge for each EPT was also checked before and after the experiment. Where multiple participants were expected to be recruited, at least five sterilized EPT stimulator probe tips, clinical marker pens, and cheek retractors were available. The sterilization of all equipment was performed following the standard sterilization policy of the Dental Hospital. Whenever possible, the contaminated EPT stimulator probe tips and cheek retractors used were packaged in the same holder to maximize and save sterilization resources. Figure 3.4 and 3.5 shows the EPT machine with its parts labelled and tip size.

Prior to the day of the experiment, a participant information leaflet (PIL) was handed to each participant to ensure the overall understanding of the experiment. On the day of the procedure, consent was gained following a full explanation of the study and the nature of the experimental procedure. Two copies of the consent form were signed by each participant; one copy was retained by the subject, and the other was attached to the participant's confidential document. This was written in the participant information sheet and also was made clear verbally. Each potential participant had an opportunity to ask any questions about the overall procedure and was also given enough time to read through the given information leaflet. Only when the potential participant felt comfortable with the information were they were asked to give consent. All participants were informed that the consent, and therefore participation, was entirely voluntary and could be withdrawn at any point without the need to give any reason. A copy of the PIL and consent form are attached (Appendices 6 and 7).

Each participant was asked to allow the investigator to hold the stimulator device against the lingual mucosa adjacent to the retromolar pad and lower wisdom tooth. Figure 3.6 demonstrates the clinical application of the machine to the lingual tissue. The strength of

stimulus was slowly increased until the subject started to experience a mild sensation around this probe tip. This sensation was explained to the patient as pinprick or tingling around the probe. When this stimulus strength was reached the probe tip was moved slowly along the mucosa in a vertical plane until the subject experienced a projected sensation to the ipsilateral side of the tongue (this movement is referred to as a ‘swipe’ in the rest of this text). At this point, the location of the probe tip was marked with a clinical marker pen (see figure 3.3). This action was repeated in several vertical planes anteroposteriorly up to the tooth in front of the wisdom tooth (mandibular second molar). Measurements were made directly on the mucosa using a customized small bendable ruler, (see figure 3.3) to give the vertical and horizontal distances from the chosen anatomical landmark.

Locating the lingual nerve in the third molar region was performed according to specific clinical landmarks in the lingual soft tissue. The three main points representing the landmark are illustrated in the following figure, 3.2, and defined as follows:

Point A - which represented the most posterior point, was location of the nerve in the retromolar pad area.

Point B - the second point represented the middle location of the nerve. The proposed location was the crown of the wisdom tooth in the case where this was erupted. In the case of full, or partial impaction, of the third molar this location was estimated as the middle area between the anterior and the posterior reference point (A and C respectively).

Point C - the most anterior point was located at the disto-lingual gingival margin of the erupted lower second molar. The following Figure illustrates the position of the points and their relation to the lingual nerve.

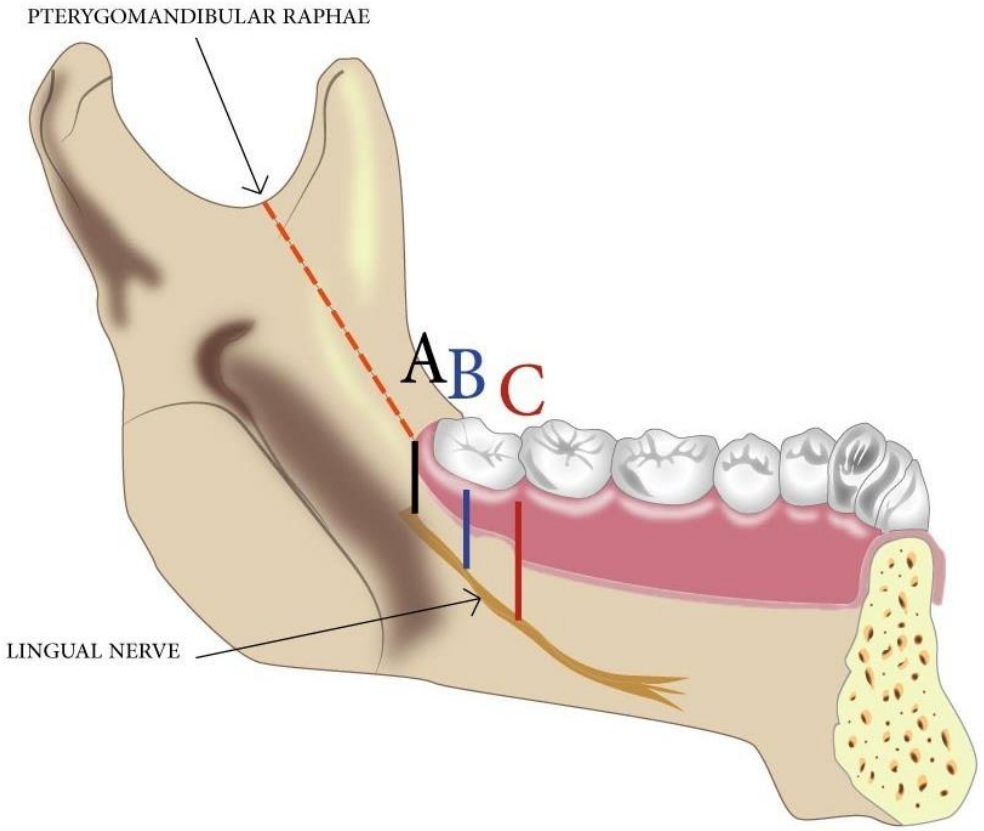

Figure 3. 2 Showing a section of the mandible in anterior-posterior position .This views the mandiular lingual side and the relation of the anatomic position of the lingual nerve in regards to the reference points; A, B and C

Clinical photographs were taken of the area marked, after identifying all or any of the three corresponding points. These photographs indicated the position of the nerve in most of the cases whenever possible. Photographic records of mapped nerves consisted of intra-oral photographs of a small area of mucosa only and therefore did not include any participant's identifiable information. Photographic images were held in the dedicated clinical camera used, and then deleted immediately after the transfer of images to the secure University central server. Each participant was (Appendix 7)

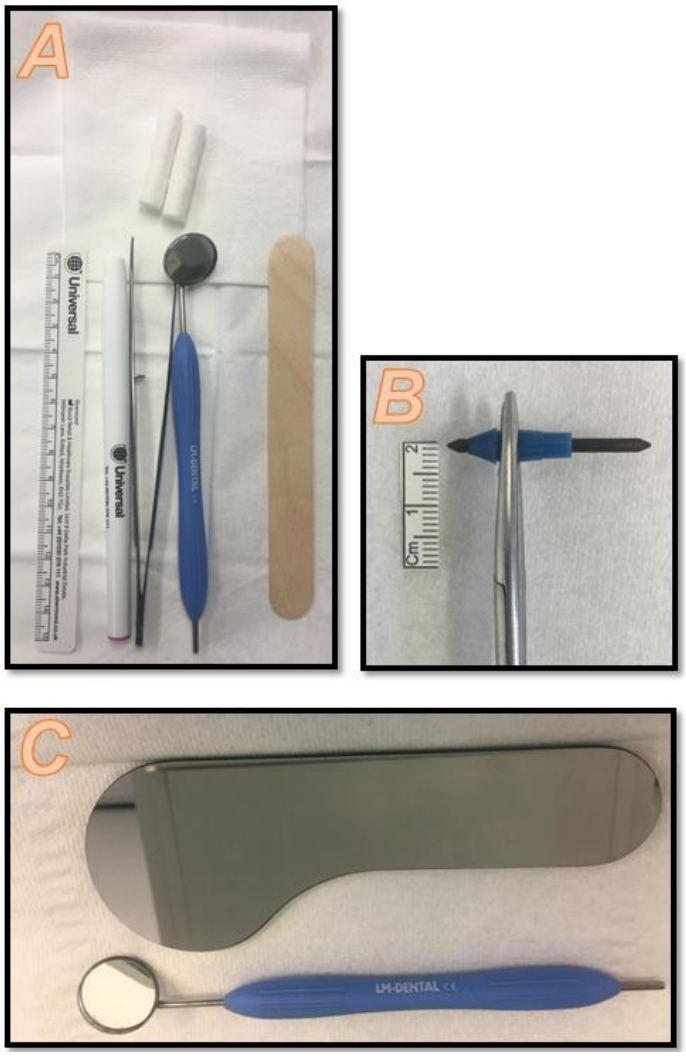

Figure 3. 3 A Showing the armamentarium used in the clinical nerve mapping experiment. B showing the tip of the intra-oral pen marker being held using mosquito forceps to enhance control and reduce bulkiness of the original plastic holder. The intra-oral ruler is trimmed and adjusted with the depth of the sulcus (20 mm). Figure 3.3 C shows both front surface mirror and intra-oral photography mirror (chromium plated) which was used in selected cases where direct photograph was difficult.

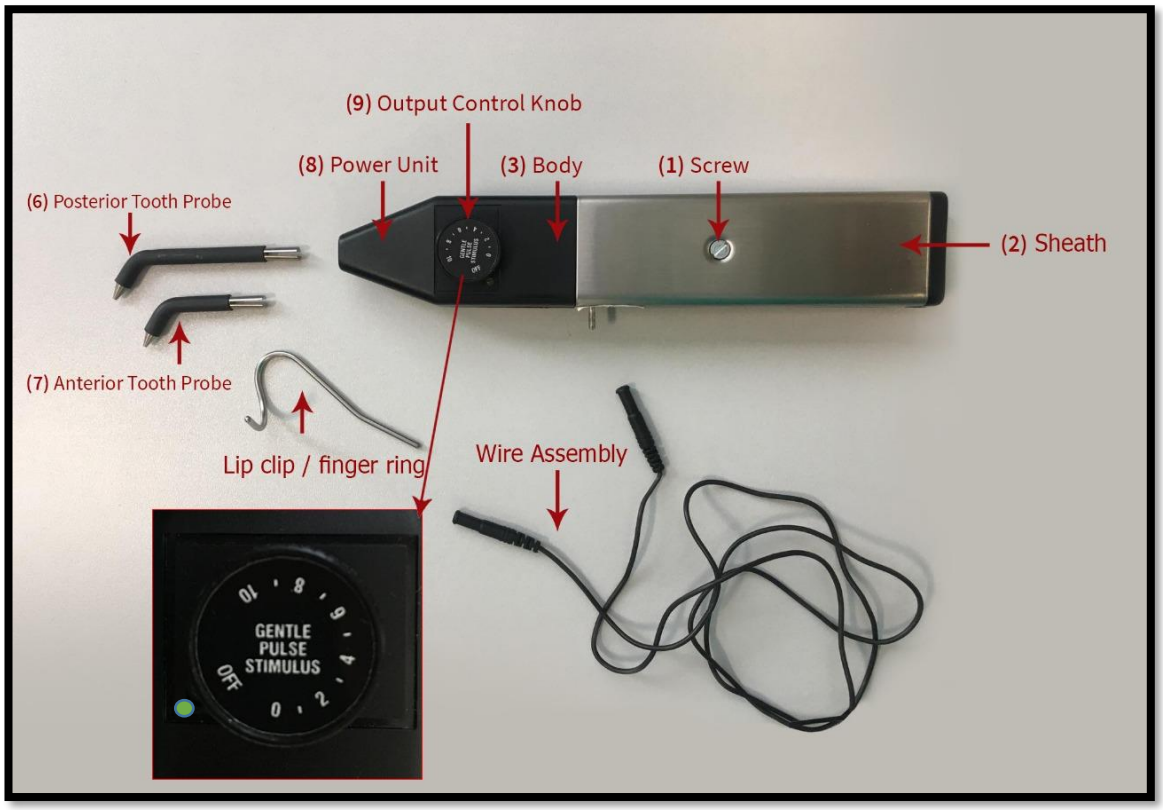

Figure 3. 4 Showing the EPT machine (Gentle-Pulse) with its labelled parts. The focused part shows the intensity controller knob with the green light that dims whenever the machine is ON. Whenever the operator turns the Knob up, the electrical stimulus increases in intensity. The machine turns off automatically after 5-6 minutes of being inactive to save the battery life.

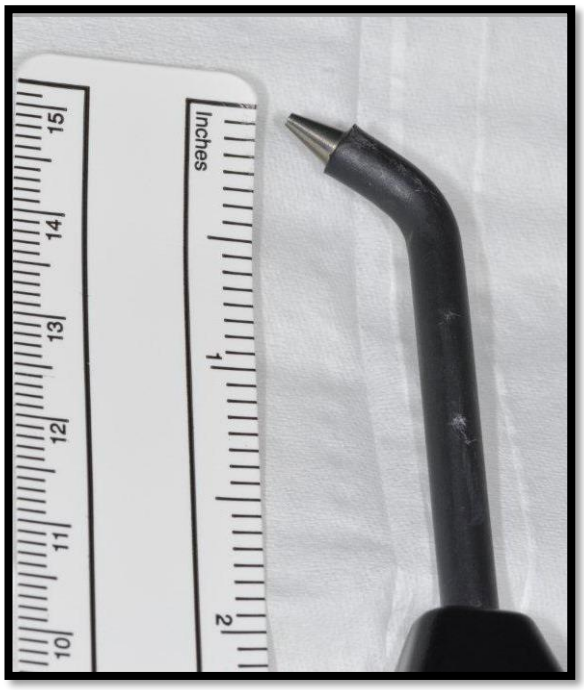

Figure 3. 5 Showing the tip size (2.5 mm) and the length of the shank of the posterior tooth probe (50 mm) that was used to map the lingual nerve in the posterior region.

Figure 3. 6 Showing EPT machine tip in the lingual surface of third molar and retromolar pad area in which point A is expected to be located.

3.2.6 Part one, Proof of concept trial:

The first part of this study was a simple descriptive proof of concept study to detect the feasibility of the mapping technique in case the electrical pulp testing was unable to detect the lingual nerve in the lingual soft tissue around the third molar. That would have been considered as a termination to the project and development of an alternative strategy would have been be considered**.** This trial also examined the methodology of trigeminal nerve stimulation using mucosal stimulation with a proprietary electrical pulp tester and assessed if it could reliably and reproducibly detect the location of the lingual nerve as assessed by eliciting a projected sensation in the distribution of this nerve. The participants' feedback during the mapping technique was considered critical as, in order to state that the nerve was identified, participants should have reported projected sensation around the lateral surface or the tip of the tongue. In those participants the corresponding points of each activated nerve were allocated and measurements taken. In participants where no projected sensation was reported on the tongue, a second operator tried to map the nerve to identify whether this was a procedural error or whether no stimulation was actually produced by the equipment.

3.2.7 Part two: An extension of recruitment study to investigate the *in-situ* position of the lingual nerve mapping using EPT.

The second part of the study expanded to target 30 new subjects to determine the prevalence of 'high-risk lingual nerve positioning' in the sample and map the nerve position around the third molar area. As described in the literature review, the lingual nerves considered to be at highest risk of damage during surgery are those at, or above, the height of the lingual alveolar bone crest. Although this correlation has not been directly proven by experiment, as no method of lingual nerve *in-situ* assessment is presently used; it was logical to assume that this positioning leaves the nerve at risk of direct trauma during mucosal manipulation as it is not protected by bone.

This position i.e. the height at, or above, the lingual alveolar bone crest was used as a surrogate for high-risk lingual nerves. According to the concept of biological width, the alveolar bone crest is measured as 2mm below the level of the lingual gingival tissues (Gargiulo *et al*, 1961). The area of interest in this study was the second molar teeth and extrapolated posteriorly to the region of the third molar and retromolar pad area.

3.2.8 Data collection:

The collected data was composed of the vertical distance of each point from the addressed reference points A,B,C and the horizontal postero-anterior distance between the first and the last point horizontally (A-C). Both right and left side nerve mapping data were distinguished from each other as was the reading of the electric pulp testing achieved in each participant. Participant feedback about the procedure, and the machine itself, was also taken into consideration after each experiment to assess the acceptability of the procedure from a participant point of view. The questions were designed to have a standardized answer that could be transferred to either 'yes' or 'no' or giving a number on a

visual analog scale (VAS). This data collection sheet was used for both the first and the second phase. The data collection sheet was printed and data entry was performed on a excel spreadsheet after each participant. The Data Collection form is attached in Appendix 8.

3.2.9 Statistical analysis:

The collected data for the first and the second phase of the study were analysed using IBM SPSS® software after exporting the excel data sheet and identification of the variables and their meaning to get the right statistical analysis. The aim was to report the descriptive statistics of each point and measure the frequency of the measurements.

3.3 Results:

3.3.1 Selected cases with an identified lingual nerve in different vertical heights:

Figures 3.7 to 3.10 shows a selected sample of mapped lingual nerve in this study. the purple dots remark the position of EPT tip in which participants had reported sensation on their tongues. Note the variable height of the lingual nerve as it seems superficial in 3.7 figure and goes deeper in 3.8-3.10.

Figure 3. 7 Representative Intra-oral photograph showing a left side mapped lingual nerve in an impacted third molar region. Notice point A, B and C with a relatively shallow vertical height.

Figure 3. 8 Representative Intra-oral photograph showing a left side mapped lingual nerve in a fully erupted third molar. Notice point A, B and C with relatively intermediate vertical height.

Figure 3. 9 Representative Intra-oral photograph showing a left side mapped lingual nerve in a fully erupted third molar. Notice point A, B and C with relatively Deep vertical height.

Figure 3. 10 Representative intra-oral photograph showing a reflection of intra-oral mirror of the right side mapped lingual nerve. Note the partial eruption of the third molar. In cases were direct photograph of the area was difficult, an intra-oral mirror was used.

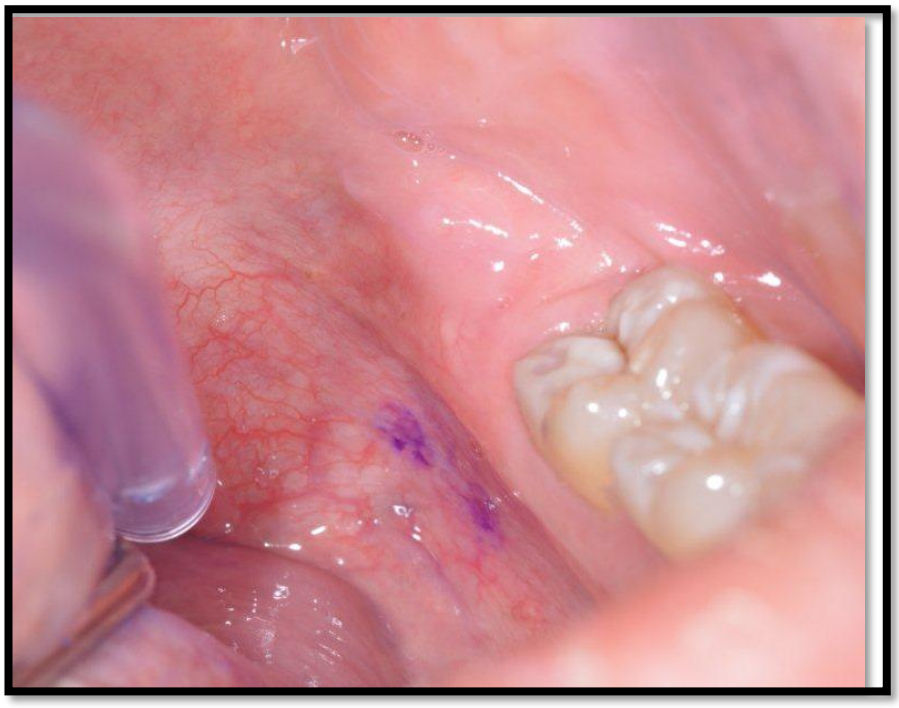

Figure 3. 11 Representative intra-oral photograph of the left side showing mapped lingual nerve. Only B and C points were able to be identified in this case,

3.3.2 Demographic data of the study population in the first study:

A total of 50 healthy participants of which 28 were female and 22 male responded to the email, poster or post-lecture briefing. Most of the recruited participants were undergraduate students at the University of Liverpool. The ethnic origin of the study participants was mainly white British with a minority of other races including African, Arabic and Asian. The detailed demographic distribution of the study was shown in table 3.1 as follows:

Demographic data	***Distribution***
Gender	
Female	28
Male	22
Age range	
18-28	36
28-38	14
Ethnic origin	
White British	28
Asian	17
Arabic	2
African	3
Total	50
Status of the third molar in both right and left side of each participant.	
Erupted	37
Partially erupted	29
Unerupted	30
Extracted/Decoronated	4

Table 3. 1 Showing detailed demographic distribution of the studied sample with the status of the third molar. The proportion of male to female in the study was closely

distributed. Most of the participants are in the age between 18-28 with almost the half were white British.

- **Recruitment chart of the first study:**

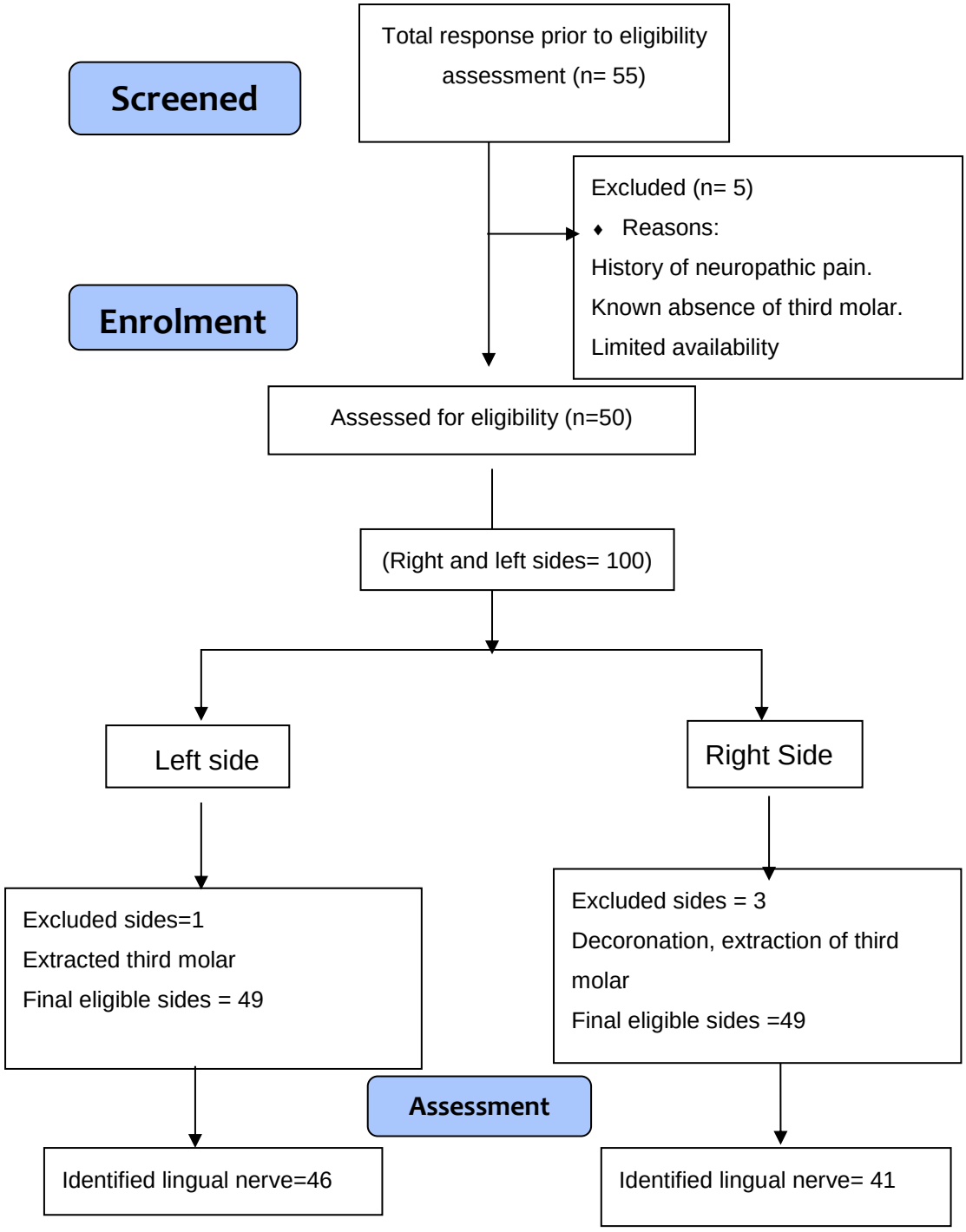

Figure 3. 12 Recruitment chart of the first and second part of the study. 55 participants were recruited to participate in the first and the second phase of the study but 5 participants were excluded due to not meeting the inclusion criteria. The 50 participants were included in the study and the lingual nerve mapping was carried out using EPT on both sides. Four sides out of 100 in the 50 subjects did not meet the inclusion criteria, which was mainly due to the known absence of the third molar - either due to extraction or decoronation Out of 50 participants 96 nerves were mapped. 87 nerves were identified with EPT as 9 were not able to be located.

3.3.3 Results of the first part of the study regarding the ability to stimualte the lingual nerve.

The experiment was trialed in 20 healthy subjects. 18 participants out of these 20 trial subjects reported a positive tingling sensation around the ipsilateral side of the tongue. Once the concept was proved in this sample, the project was expanded for the full recruitment plan. Figure 3.13 demonstrates the proportion of participants with positive response to EPT.

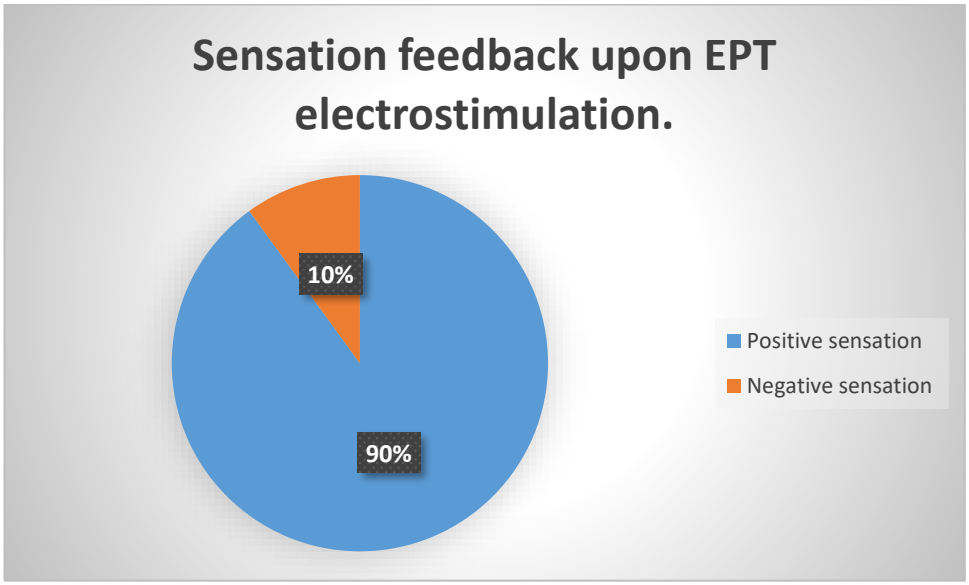

Figure 3. 13 Pie chart showing the percentage of people with positive sensation on the ipsilateral border of the tongue as a result of electrostimulation of the lingual nerve. Only 2 participants (10%) of the first part study did not have any sensation around the tongue of the activated side.

3.3.4 Descriptive analysis of mapped nerves and the vertical height measurements.

The total number of sample is considered to be nerves rather than individuals. Thus, the excluded sides are not necessarly an excluded participants as still one of the sides was elidgible for recruitment. The left side lingual nerve identification was slightly higher than the right side. See table 3.2.

Category/Sides	***Frequency right side***	***Frequency left side***	***Total number of sides***
Full recruited	50	50	100
Included side	47	49	96
Excluded side	3	1	4
Identified lingual nerve	41	46	87

Table 3. 2 Indicating number of included and excluded sides in the whole sample and the number of identified nerves in the first and the second part of the study.

Upon mapping the lingual nerve, the position of the nerve was assessed in three different locations which were decided to lie on the fixed landmarks. In some of the cases it was not possible to allocate all of the points (A, B and C). Table 3.3 shows frequency of valid and missed data for each point and the mean value with standard deviation for each point. Note that the mean values of points in the right side are relatively close to the left side of all points.

Descriptive statistics /Points of the mapped lingual nerves	***Point A***	***Point B***	***Point C***
Identified right	36	34	37
Identified left	37	41	42
Total identified	73	75	79
Total Unidentified	23	21	17
Total sample	96	96	96
Mean value of the vertical height right	9.55mm	10.44mm	12.40mm
Mean value of the vertical height left	9.72 mm	11.04 mm	12.28 mm
Mean value of the total height	9.64 mm	10.77 mm	12.34 mm
Standard deviation right	2.65 mm	2.38 mm	3.32 mm
Standard deviation left	3.30 mm	3.04 mm	3.04 mm
Standard deviation total	2.98 mm	2.76 mm	3.16 mm
Number of points less or equal to 5 mm	5	2	1

Table 3. 3 Describes the detailed results of point A, B, C and their descriptive analysis on both right and left sides of the total sample size.

The measurements taken for each point were measured in millimeters using a standardized intra-oral ruler. The following tables (Tables 3.4 – 3.6) describe the frequency of each measurement in each point.

Vertical height in mm Point A in mm	***Frequency***	***Percentage of total nerves identified at point A. with the corresponding height.***
4 mm	1	1.4 %
5 mm	4	5.5 %
6 mm	2	2.7 %
7 mm	9	12.3 %
8 mm	14	19.2 %
9 mm	10	13.7 %
10 mm	13	17.8 %
11 mm	3	4.1 %
12 mm	2	2.7 %
13 mm	9	12.3 %
14 mm	1	1.4 %
15 mm	2	2.7 %
16 mm	1	1.4 %
17 mm	0	0 %
18 mm	1	1.4 %
19 mm	1	1.4%
Total	73	100%

Table 3. 4 Showing a detailed frequency of measurements of point A measurements and their percentages amongst the study sample.

Vertical height in mm Point B	***Frequency***	***Percentage of lingual nerves identified at point B. with the corresponding height.***
4mm	0	0%
5 mm	2	5.5%
6 mm	2	2.7 %
7 mm	2	12.3 %
8 mm	2	17.8 %
9 mm	16	13.7 %
10 mm	23	19.2 %
11 mm	3	4.1 %
12 mm	8	2.7 %
13 mm	4	12.3 %
14 mm	4	1.4 %
15 mm	5	2.7 %
16 mm	0	1.4 %
17 mm	2	0 %
18 mm	2	1.4 %
Total	75	100%

Table 3. 5 Showing a detailed frequency of measurements of point B measurements and their percentages amongst the study sample.

Vertical height in mm Point C	***Frequency***	***Percentage of total nerves identified at point C. with the corresponding height.***
4 mm	1	1.3 %
5 mm	0	0 %
6 mm	2	2.5 %
7 mm	1	1.3 %
8 mm	4	5.1 %
9 mm	5	6.3 %
10 mm	12	15.2 %
11 mm	4	5.1 %
12 mm	12	15.2 %
13 mm	12	15.2 %
14 mm	5	6.3 %
15 mm	11	13.9 %
16 mm	3	3.8 %
17 mm	1	1.3 %
18 mm	5	6.3 %
19 mm	0	0%
20 mm	0	0 %
21 mm	1	1.3 %
Total	79	100%

Table 3. 6 Showing a detailed frequency of measurements of point C measurements and their percentages

In the previous three tables, the distribution of the measurements and their frequency at each point follow the normal distribution of the nerve posterior shallower anterior deeper. Note the highlighted measurements in each table which represents the higher and the lower measurement of each point. The following figure, 3.14, illustrates that more clearly.

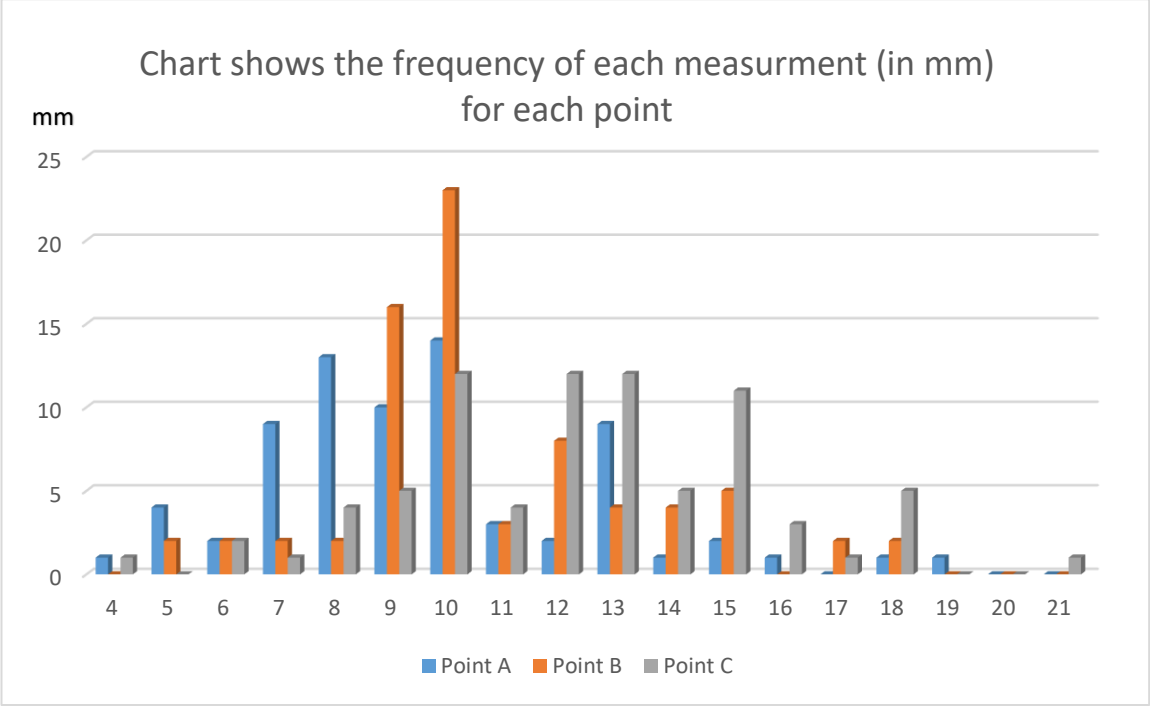

Figure 3. 14 Comparison of the measurements (in mm) between the three points A, B, C.

3.3.5 Descriptive analysis of the horizontal distance A-C.

Following identification and mapping of the lingual nerve, additional information regarding the horizontal distance between the retromolar pad area and the attached gingivae of the distal of the second molar tooth was measured (see table 3.7). This was performed to estimate the horizontal distance of the studied area of nerve position.

Descriptive value	***Right side A-C Horizontal distance***	***Left side A-C horizontal distance***
Number of sides	46	47
Mean distance	13.39 mm	13.57 mm
Standard deviation	± 2.5	±2.8
Minimum value of A-C	8 mm	8 mm
Maximum value of A-C	20 mm	20 mm

Table 3. 7 Showing the horizontal distance between the references points of the mapped nerves.

The electric pulp tester used has nine different grades for voltage control. Throughout the procedure the device was adjusted to different levels of voltages according to the participant's individual thresholds of sensations and the tissue resistance. The mean value of the stimulus voltage was 6.63 ± 1.41 standard deviation. Table (3.8) illustrates the values of the EPT voltage and their frequency.

Value of EPT stimulus intensity	***Frequency***	***Percentage***
3.0	1	1 %
4.0	7	7.3 %
4.5	1	1 %
5.0	11	11.5 %
5.5	0	0 %
6.0	17	17.7 %
6.5	4	4.2 %
7.0	30	31.3 %
7.5	0	0 %
8.0	20	20.8 %
9.0	2	2.1%
10.0	3	3.1 %
Total	96	100%

Table 3. 8 Showing the distribution of EPT stimulus intensity value and its frequency amongst the tested participants.

3.3.6 Participant-reported feedback:

Unpleasant sensation:

At the end of the lingual nerve mapping, each participant was asked a few questions to describe their experience of this technique and to reflect on the whole procedure. Each participant was made aware that the answer was preferred to be a number on a 0-10 scale where number 0 denoted no pain or discomfort, and number 10 meant it was highly painful or uncomfortable. The following table (Table 3.9) shows the reported feedback from participants on the postoperative questions:

Unpleasant sensation reported by participants from the EPT	***Mean (0-10 scale)***	***Minimum***	***Maximum***
Discomfort from EPT tip on the attached mucosa.	2.9	0	7
Discomfort from EPT tip on the non-attached mucosa.	4.6	0	8
Discomfort of the electric stimulus of EPT.	2.8	0	7

Table 3. 9 The mean, minimum and maximum values of each question asked to every participant at the end of the procedure. Note the discomfort sensation at the non-attached gingivae was reported to be the highest. The electrical stimulus reported to be accepted by most participants with minimum discomfort, 2.8.

Adverse events:

Adverse events are unexpected problems that arise during a procedure or experiment. In the lingual nerve mapping technique, any adverse events were recorded, as the participants had the chance to express them in their own words. Any lasting sensations experienced in the oral cavity, following the procedure, were also recorded at the end of the experiment. The reported adverse sensations and lasting sensations are reported in the following tables (Table 3.10 and table 3.11):

Reported transient adverse events	*Number of participants*
• **No reported adverse event**	15
• **Bad taste from the intra-oral ink**	4
• **Dental pulp electrical stimulation with EP**	4
• **Pressure in the floor of the mouth from retraction of the tongue**	10
• **Sharp sensation at the floor of the mouth**	4
• **Vibratory sensation of the floor of the mouth (motor muscle activation i.e. Nerve to mylohyoid)**	3
• **Pins and needles/tingling sensation around the tongue**	2
• **Discomfort or controlled gag reflex**	4
• **Dryness of the mouth secondary to the salivary ejector**	3
• **Total number of participants**	50

Table 3. 10 Transient adverse events from participants' experience of the experimental technique. This can be related to any armamentarium used in the technique. Note that the pressure on the floor of the mouth counted for the most

reported event, followed by the taste of the ink, gag reflex and accidental pulp stimulation using the EPT probe tip.

Lasting sensations:

Duration of any reported lasted sensations following EPT stimulation(i.e. tingling, vibrations, numbness or pins and needles)	***Number of participants***
• **No identified lasting sensations**	38
• **Lasting sensation less than one minute**	8
• **Lasting sensation more than one minute (up to seven minute)**	3
• **Lasting sensation up to 3 days**	1
• **Total number of participants**	50

Table 3. 11 Duration of experienced lasting sensations amongst the participants. Most of the participants didn't report any significant lasting sensation apart from one participant which was thought it could be related to muscle spasm due to prolonged retraction of the tongue during the lingual nerve mapping on the left side.

3.4 Discussion:

3.4.1 Introduction, findings of the first part of the study:

The research aims and objectives of the first study with its two parts were achieved. In the first part, most participants (90%) were able to identify the tingling sensation on the lateral border of the tongue on the same side of the mapped area. This was considered to be enough to indicate a proof of concept. The suggested criteria for success was taken as the satisfactory location of the nerve in at least 80% of subjects were identified, based on the usefulness sensitivity test (Lalkhen & McCluskey, 2008). In the unlikely event that the concept had not been applicable, an alternative project to define the ideal stimulation parameters for reproducibility was going to be considered. That was suggested as considering different electrostimulating parameters to generate impulses that could activate the lingual nerve.

3.4.2 Demographics and their relation with the study findings:

Most subjects recruited in the study were between the ages of 18 to 28 y. This age group is considered representative of the population who have their third molar extraction as frequent oral surgical procedure. According to the recruitment criteria, this group of participants was found to be convenient to participate and fulfill the aims and objectives of this feasibility study as a ‘convenience sample’

The homogenous distribution of the genders in this study were considered to be reasonably close to the actual distribution in the general population. This has a positive impact as the nerves were mapped in males and females in a relatively equal proportion. Where each participant had both right and left lingual nerves mapped that has almost doubled the collected data, thereby increasing the overall sample size. Although half of participants were white British, in an age group that ranges between 18-28 years old, this allowed the other recruited half to be from other ethnic backgrounds. This has supported

the sample to be more representative of the general population unlike most of the cadaveric studies that are either limited or not specified populations and without information regarding the oral condition, the status of the third molar was identified to be included in this study. (Behnia et al, 2000; Benninger et al, 2013; Erdogmus et al, 2008; Kiesselbach & Chamberlain, 1984; Kim et al, 2004; Pogrel et al, 1995; Sittitavornwong et al, 2017)

3.4.3 Identifying the lingual nerve using EPT, challenges, drawbacks and how they were overcome

The pre-operative assessment of the clinical *in-situ* position of the lingual nerve has not been investigated previously in the literature. The developed method in this current study adopted a different approach in trying to identify the position of the lingual nerve, in that an electrical stimulation device was used to locate the nerve position in an effort to identify nerves in vulnerable positions. Electric nerve stimulation is a well-established procedure that has been used to identify peripheral nerves prior to various surgical and medical procedures but most of the reported literature was on stimulation of extra-oral nerves rather than intra oral sensory nerves (Bo et al, 2015; Sippel & van Zundert, 2012).

As the name implies, EPT was developed to investigate the sensibility of the nerves in the dental pulps. Hence, the tip is designed with a flat-end surface which needs to be applied steady and flat to the surface to provide maximum contact with the tooth tissue in order to transmit electrical impulses (although this can be improved by use of a conducting gel on the probe). This type of apparatus has also been reported and used safely on soft tissue with no potential soft tissue damage (Dal Santo et al, 1992). Following its use on soft tissue in the current study, it was noted that the ability to place the flat probe surface on the soft tissue, to maximise the contact with the lingual soft tissue, was difficult due to the irregularity of the lingual plate and the nature of the overlying tissue.

The bulkiness of the machine handle also limited the ‘swiping’ movement of the tip intraorally. A tip with a longer handle was used

subsequently to overcome the limitations in movement as well as getting a better field of view. Although the tip surface was considered to be relatively smooth, the fine edges were reported by some of the participants to cause discomfort and irritation whenever the tip was advanced deep in the non-attached mucosa of the lingual tissue and the retromolar pad area. This is explained by the fact that the nature and the texture of the tissue in those areas is quite thin and not keratinized. An innovative improvement to the tip design of the EPT could overcome this problem. Adopting a 'ball-ended' tip design that could be 'swiped' along the tissue easily, with rounded margins in the study area should improve patient comfort without reducing diagnostic yield.

A 9- volt battery operated EPT apparatus is convenient to use as it doesn't need to have a wire connecting it to an electrical source while being used and this would provide freedom of movement. A battery check was undertaken prior to its use for every single participant by switching on the machine and test the LED indicator. In addition, this machine also offers a battery-saver mood which automatically turns off the machine after 5-6 minutes of non-use. The current in this machine is constant. Therefore, the intensity of the current in the machine is unlikely to be affected by the status of the battery. In fact, the power will cut off completely if the battery is low rather than providing weak current. This is of great importance in the clinical application as the nerves were mapped using the same machine, a standardised current is important to ensure applying the same conditions in all the subjects.

One of the most noticeable drawbacks of the design of the apparatus used in this study was that it didn't have an audible alert to inform the operator that it was switched off. This was noticed during the experiment, especially whenever a delay was required to map the nerve, and the EPT turned off leaving the participants feeling nothing but pressure. Frequent checking every time the tip was introduced to the area being tested was ensured to prevent this problem from arising.

Marking each point using an intraoral pen was also challenging. As with any regular pen, the straight handle reduced the flexibility of to

reach the posterior lingual area. This was resolved by removing the ink tip from the pen holder and attaching it to mosquito forceps to facilitate accuracy control. Since the mucosal lining and the attached gingivae was always coated with serous or mucous saliva the diffuse distribution of the ink following point marking made the visualisation of the main point difficult in that limited area. To reduce the impact of those challenges, moisture control was ensured using sterile gauze while performing tongue retraction, as well as use of a saliva ejector to prevent pooling of the saliva in the lingual sulcus. The tip of the marker pen was also chosen to be as narrow as possible. By following the instructions for use, the pen head was applied perpendicular to the soft tissue in order to ensure fine lines of almost 0.25 mm in diameter, according to the tip size.

The measurements were taken by the same operator after mapping the nerve in the first and second phase using a small intra-oral small of 20-25 mm in length. This length was adjustable and trimmed according to each participant's depth of lingual sulcus and this ruler was adapted to the lingual mucosal surface to measure the distance between the identified point and its corresponding reference landmark.

Since the lingual surface of the mandible has an irregular surface due to the shape of lingual crest (which can cause bulbosity of the area) and the attachment of the mylohyoid muscle, the visualisation of the assessed point and taking the measurements became difficult. In order to improve this, a flexible intra-oral ruler was used. This facilitated the adaptation of the ruler to the lingual surface to give more accurate measurements. The use of an intra-oral dental caliper was suggested early in the study to improve accuracy and consistency of the measurements but other drawbacks of this tool, such as pointed tips which could irritate the soft tissue, and its bulk confirmed the decision to use the intraoral flexible ruler.

In most of the cases, intra-oral photography was taken to provide an overview of the mapped nerve in that area and to show the cases where the mapped nerve was, relatively, superficial in position. The ability to have perpendicular access to the field of view was hard due to

the position of the tongue and the limitations of mouth opening. Some of the participants had restricted mouth opening and also a prominent lingual crest which made observation of the mapped lingual nerve, and adapting the tip to lie flat on the lingual tissues difficult. To overcome this, an intra-oral mirror was placed vertically and a mirror reflection photo of the site was taken. In some the cases, even using these techniques, intra-oral photography was difficult due to limited accessibility, visibility and gag reflex.

Previous descriptions of the lingual nerve position and anatomy have mainly been reported following cadaveric dissection studies (Hölzle & Wolff, 2001; Kim et al, 2004; Mendes et al, 2014; Pogrel et al, 1995; shinora et al, 2010). All these studies have reported the exact position of the nerve following direct observation of the nerve tissue. However, by comparison, in the present study, the position of the nerve was assessed indirectly, and *in vivo*. This was elaborated by activation of the nerve and assigning the points in which the activated lingual nerve emitted certain sensations on the lateral surface of the tongue. Prior to the procedure, as part of gaining valid consent, participants were informed of the different types of sensations expected around the third molar and the tongue. Hence, the results that were obtained were reliant on the subjective feedback from each participant which might have increased the risk of presentation bias.

Whenever the lingual nerve was mapped, it was expected to identify the three points alongside its distribution. Due to the unpredictability of finding the three points in each mapped lingual nerve, it was accepted that at least one point had to be found In order to mark the lingual nerve as identified. EPT was re-applied to the identified point to ensure consistent measurements collecting the positional data.

3.4.4 Critical appraisal of the lingual nerve position in the literature and its relation to the current study:

Comparing the results of the current study with the anatomic cadaver and imaging studies complicates interpretation. This could be due to differing methodologies as follows:

A) The relation between the nerve and the anatomical landmark in the study and the study of the nerve in different intersections.

B) The potential distortion of the nerve and the surrounding tissue as a result of fixation methods (in cadaveric studies), this can lead to over-drying of nerve tissue as well as displacing the nerve from its actual *in vivo* location.

C) Differing results as a consequence of the use of different measuring techniques i.e. calipers(Behnia et al, 2000) vs radiographic measuring(Karakas et al, 2007) vs digitalizing methods(Mendes et al, 2014).

D) The difference in sample size in each study.

E) Different populations studied.

In this study the lingual nerve was investigated to establish the vertical relationship to the retromolar pad area, the lower third molar and the second molar. Most cadaveric studies have investigated the relation of the third molar at only one point apart from (Kim et al, 2004), who investigated the position of the nerve in three different places, and (Chan et al, 2010) who examined the nerve more anteriorly, in the second molar region. In the current investigation, the criteria of nerve positioning in order to identify whether the nerve is at high-risk position, a vertical distance of any point which was at or less than 5 mm was the main indicator of its vulnerability. This is explained by the fact that the allocated point on the stimulated nerve might be drawn on the inferior or middle border of the nerve. From the studied literature, the diameter of the lingual nerve ranges from 1.86mm (Kiesselbach & Chamberlain,

1984) to 3.45mm (Pogrel et al, 1995). By considering the intermediate diameter, which is almost 2.5mm, and adding the sulcular depth to the biological width of -1.91 to 2.07mm (Gargiulo et al, 1961; Vacek *et al*, 1994), 5.04 to 5.21mm should be considered as the minimum distance where the nerve can actually be positioned, at or just above the lingual bone crest. See the illustration below (Figure 3.15) for clarification:

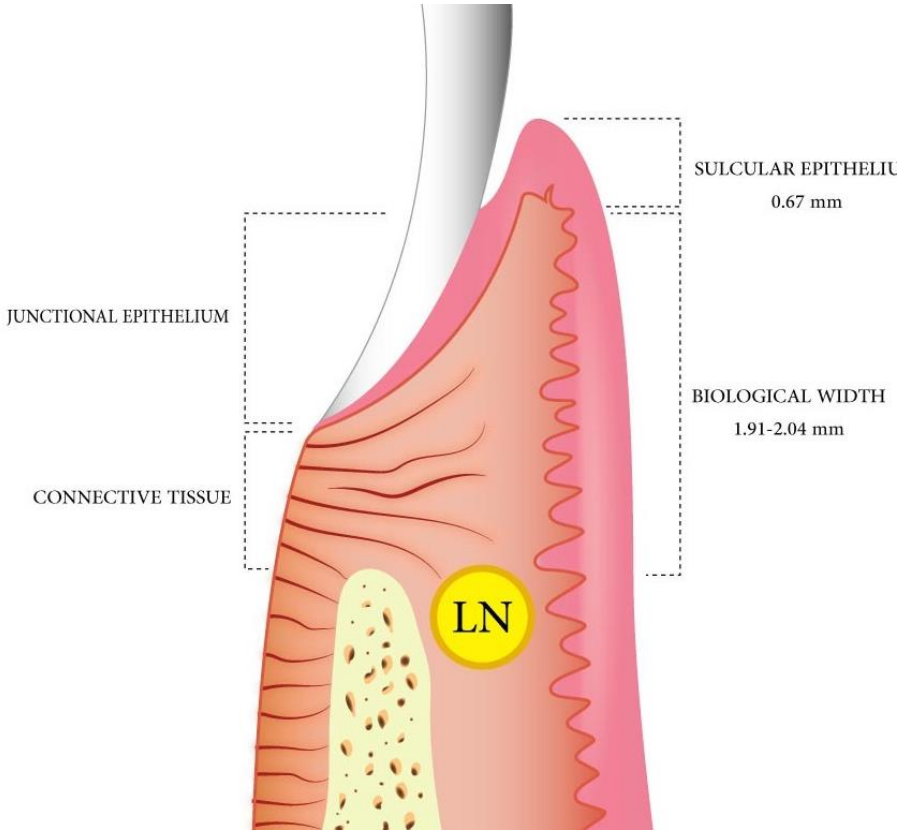

Figure 3. 15 Illustrating the position of the lingual nerve in relation to the soft tissue. in case the lingual nerve was at the level of alveolar bone crest, the addition of biological depth to the sulcular epithelium with the estimation of the diameter of the lingual nerve (average of 2.5mm) will be between 5.07 to 5.21mm (Vacek et al, 1994).

In the cases where the lingual nerve was not able to be identified, it was unclear whether the nerve was embedded deeper in the lingual mucosa or the participant had a high threshold of nerve activation beyond the electric current of EPT. In the participants, Point A was the most unidentifiable point amongst the three (73 out of 96). This may be due to the increased thickness of soft tissue around that area compared to point B and C. It is possible that the tissue thickness may have contributed to reducing the intensity of the electric stimulus to activate the nerve. The detailed data of the precise positioning of the lingual nerve within the soft tissue (impaction) has not been fully investigated. Most cadaveric and imaging studies have however looked at the horizontal relationship between the lingual nerve and the underlying bone.

Although the detection of this relationship is important, since close contact of the nerve with the bone can leave the nerve at higher risk of injury during surgical extraction of the third molar. It is still of high importance to know the depth of the nerve in the soft tissue around the third molar area. There is little evidence that nerve depth in soft tissue has been investigated in cadaver studies. In 2001, a dissection study measured the horizontal distance from the lingual nerve to the lingual soft tissue of the retromolar pad area. The mean value of nerve depth in the soft tissue was 4.41±1.44 mm (Hölzle & Wolff, 2001). Garbedian in 2010, however, observed that the nerve was deeper within the soft tissue of the retromolar pad area (9.22 ±1.72 mm) (Garbedian, 2010).

The large difference between these studies is probably related to the differences in the measurement's landmarks, the small sample size as well as the difference in the methodologies and soft tissue handling after fixation.The depth of impaction of the lingual nerve through soft tissue is of significant importance. Flap incision and retraction can increase the risk of the lingual nerve damage if the nerve was positioned superficially in the soft tissue around the retromolar pad area (Pichler & Beirne, 2001). This is also recognized as one of the

limitations of the current investigation, as this kind of information is not possible to be provided via EPT nerve mapping.

3.4.5 The vertical distance of the lingual nerve in comparison to the previous measurements in the literature:

Interpreting our results with the corresponding findings in the literature was performed carefully. Since, in our current study, reference points were measured from from the soft tissue margin, an extra 2-3 mm should be added to compensate for the biological width. The following section of the text discusses the detailed findings for each point, and compares these with the data already available in the literature.

1) Retromolar pad area: Point A

Point A represents the most posterior, and the first point where the lingual nerve was investigated. This was allocated just in the soft tissue anterior to the insertion of pterygomandibular raphe, where the lingual mucosa is in a point of transition from a vertical to horizontal direction. The mean value of the vertical distance between point (A) and the corresponding soft tissue landmark was calculated to be 9.64 ± 2.90mm.

These findings are relatively close to the results found in found in two previously published cadaveric dissection studies (Kim et al, 2004; Pogrel & Goldman, 2004). Pogrel et al (1995) reported the mean vertical distance between the lingual nerve and the crestal bone around the retromolar pad area to be 8.31± 4.1mm, and Kim et al, (2004) at 7.8 mm. It is worth adding that both of these studies had less than half of the sample size of the current study, with an entirely different methodological approach which may explain the differences in the findings. Although the title of the Pogrel et al (1995) paper is the 'Investigation of the nerve position in the third molar', a detailed review of the paper showed that the presented measurements were actually taken from the retromolar pad area.

2) Middle of the third molar: Point B

In the current study, point B represented the relation of the lingual nerve to the middle of the third molar. 75 points out of 96 were able to be identified. The mean value of this point amongst the sample of the current study was 10.77 ± 2.7mm. This is almost one millimetre deeper than point A which represents the normal course of the lingual nerve. As it passes towards the tongue it tends to descend deeper in the lingual tissue.

These results lies in close relation to what has been found by a number of other investigators - 7.84±1.65 by (Hölzle & Wolff, 2001) and 9.56±5.28 (Karakas et al, 2007). The large standard deviation of the Karakas et al paper can be attributed to the limited sample size. In contrast, other reports in the literature were found to be different to our findings. Behnia et al (2000) noted that the mean vertical distance of the nerve was 3.06±0.42 mm and this in close agreement with both Kiesselbach & Chamberlain (1984) and Miloro et al (1997) who found that the vertical distance was 2.28±1.96 mm and 2.75±0.97mm respectively. The possible reasons for this significant difference in those studies compared to the current one are 1) smaller sample size (Kiesselbach & Chamberlain, 1984; Miloro et al, 1997); 2) the possibility of a distorted position of the nerve as a result of tissue fixation, the status of the cadavers (i.e fresh vs old) and 3) the use of different measuring techniques and landmarks (Behnia et al, 2000; Hölzle & Wolff, 2001; Karakas et al, 2007).

3) The disto-lingual gingival margin of mandibular second molar: Point C

In the current investigation, point C represented the distal gingival margin of the second mandibular molar. 79 out of 96 points were identified and the mean value of the vertical distance between the identified lingual nerve and the reference point was 12.34±3.1 mm.

According to the literature, our results lies between the two others which have investigated the location of the lingual nerve round a corresponding region to point C. Their findings were that the average vertical distance of the lingual nerve in the second molar region was 9.6mm.(Chan et al, 2010) and 15.5 mm (Kim et al, 2004). No further detail about the standard deviation was mentioned in those studies. As well as the lack of variability of the result, the different methodologies and small sample size can explain the discrepancy in findings between both studies.

The measurements presented in this study are one of the few which have investigated the position of the lingual nerve in three different positions. The ability to match the measurements recorded at each of these points (A,B &C) with the existing literature is quite demanding and are inconsistent with many of those due to different methodologies, aims behind the research, and also sample sizes. However, overall, our resultant measurements of the nerve, in the different locations, were found to be within the range of the other presented literature.

It is notable that the mean value of the vertical distance of point A, B, C did not differ significantly between right and left sides which is consistent with the findings of previous cadaver studies which did not find any significant differences between their measurements of the lingual nerve of both sides (Behnia et al, 2000; Hölzle & Wolff, 2001; Pogrel et al, 1995).

3.4.6 The position of the nerve as being above or below the alveolar crest:

In the current research project, the lingual nerve was identified to be above, or at the level of the alveolar crest when the measurement of the mapped point was within a certain distance of the allocated landmark. If we take into consideration the thickness of the soft tissue then we would consider the lingual nerves at a height of 5mm or less to be in a vulnerable position. We observed 5 nerves at this height at point A, 2 at point B and 1 at point C, 5.7% of the total 87 identified. According to the results from cadaver studies within the literature, our findings could be considered to have a slightly lower prevalence than expected. Pogrel et al (1995) and Miloro et al (1997) both found that the nerve lies at, or above, the lingual crest in about 10% of the total sample. Behnia et al (2000) observed that 14% (of the total 669 dissected nerves) were found to be above the lingual crest (Behnia et al, 2000). This is a relatively larger sample size than Benninger et al (2013) who identified 21% of the 28 dissected nerves to be at, or above, the alveolar crest, although that study also utilised the use of clinical ultrasonography intraorally and confirmed the ability to identify nerves at or above the lingual crest. Further details on the protocol of ultrasound usage and the position of the identified nerve were, however, lacking. In contrast, Hölzle & Wolff (2001), have reported that only 8.8% of the 68 dissected nerves are located at or above the lingual crest. Kieselbach & Chamberlain's cadaveric investigation found 17.6% of the dissected nerves to be above the lingual crest. Interestingly, their clinical direct observation of the *in-situ* position of the lingual nerve was close to our findings where they described that, out of 256 patients, the lingual nerve was seen above the alveolar crest in 4.6 % of the cases.

3.4.7 The limitations of electric stimulation of the lingual nerve in the third molar region:

Following mapping of the lingual nerve, although most of the participants reported tolerable experience with no pain or discomfort, some unexpected findings were associated with this technique. In one case, the activated nerve was reported by participants to be at different vertical levels in each point. This has led to confusion in the positional data taken from this case. The assumption case was made based on the existence of vertical accessory branches of the lingual nerve in this area (Kim et al, 2004). In clinical application, the superficial nerve would be considered the main nerve to provide safety margins for any surgical procedure around the third molar region.

The complex anatomy of the oral cavity leaves the lingual nerve in proximity with other sensory and motor nerve fibres. By swiping the EPT down the lingual mucosa, other nerve tissue can be possibly gets activated. An intermittent tension at the floor of the mouth was reported by three participants throughout the study. By understanding the relevant anatomy of the lower third molar region and the floor of the mouth, this could be explained by motor nerve activation of mylohyoid nerve (which innervates the main muscle of the floor of the mouth) that nerve runs in close proximity to the lingual nerve with possible cross innervation with the lingual nerve (Kim et al, 2004; Racz & Maros, 1981).

Chapter 4: investigating the agreement of EPT in identifying the lingual nerve in both inter-observer and intra-observer experiment: A reliability study

4.1 Introduction:

Once we had demonstrated the feasibility of stimulating and detecting the lingual nerve *in-situ* using the new technique, we then investigated whether the method of measuring the vertical height of the lingual nerve was reliable, accepting that any method of measurement is subject to some inaccuracy. However, if the method is to have practical value clinically it must be reasonably reproducible and have a usable level of agreement between: either different trials of the same operator, or different operator, towards the same sample under the same conditions (Koo & Li, 2016). These evaluations are considered fundamental to assess the confidence in the measurements taken by any tool and were therefore adopted for this investigation.

In the lingual nerve mapping technique, different factors can affect the performance of the EPT and the collected data. Those factors can be categorised as participant related factors such as excessive salivation, limited mouth opening or tongue motion which can affect the visibility of the studied field, operator related factors which can be related to EPT handling, marking the allocated point or visualising the measured point.

This reliability study was therefore undertaken to check the level of agreement between different trials, or operators, to identify whether this technique's results are reproducible and to determine whether the effect of all of the above mentioned factors are, or are not, clinically significant to the final results of this sample.

Clinical measurements fall into different types. This is important to understand as different statistical tests can be used to measure the agreement according to the type of measurements. For instance, categorical data is known as a qualitative data that can be further classified into

1.Nominal or nonordinal
2.Ordinal scale (e.g. fair,mild,serious, critical or life-threatening) and
3.Binary outcomes (yes /no or normal/not normal).
The Cohen kappa and weighted kappa are some of the designed tests to analyse binary and ordinal categorial data (Koo & Li, 2016; Lin *et al*, 2012; Watson & Petrie, 2010) .

In contrast, continuous data are known as quantitative data and can be categorized further into interval or ratio data. In this current study, our collected measurements of each point A, B, C. were considered to be continuous ratio data and measuirng the agreement of this type of data is normaly carried out utilising intra-class correlation coefficients (ICC).

4.2 Aims:

This study was conducted to:

1. Investigate the level of agreement in measurements taken by two different operators using the same EPT machine under the same clincial conditions to determine the external validity of the technique.

2. Investigate the level of agreement in measurement between two different measurements recorded by the same operator on two different occasions to determine the internal validity of the technique.

4.3 Methodology:

This study was carried out in parallel to the second part of the first study. Participants who had their lingual nerve mapped in the first study were asked to participate again for this second part. The participation was entirely voluntary and further explanation of the procedure was given via an information leaflet and new consent gained. A £10 voucher was also offered by the end of the session as a reimbursement for each participant.

10% of the overall sample size (96 lingual nerves) was included in the study. This sample size was based on the number of the lingual nerves rather than the participants. For both intra-observer and inter-observer reliability, at least 10 lingual nerves were mapped in at least 8 different participants. Whenever possible the recruited participant had their right and left lingual nerves mapped and the new measurements were recorded. Each side was recorded by a different operator to compare the measurements with the original ones. However two participants had their both right and left side mapped by the same operator in each experiment due to the non-availability of the second operator at time of examination.

4.3.1 Inter-operator agreement:

The same clinical procedure for lingual nerve mapping was conducted again using the same EPT, disposable intra-oral pen marker and the adjusted flexible ruler. There was at least two week between the first and the second lingual nerve mapping between different operators. The new measurements of point A, B, C were recorded on a different data collection form by a third observer (trained dental nurse). Out of 9 participants, 11 lingual nerves were mapped by the second operator to investigate inter-operator agreement.

4.3.2 Intra-operator agreement:

The same clinical procedure for the lingual nerve mapping was conducted again using the same EPT, disposable intra-oral pen marker and the adjusted flexible ruler. There was at least two week between the first and the second lingual nerve mapping by the same participant. The new measurements of point A, B, C were recorded on a different data collection form by a third observer (trained dental nurse). Out of 8 participants, 10 lingual nerves were mapped by the same operator to investigate intra-operator agreement.

4.3.3 Statistical analysis:

ICC is available in 10 different forms, each form has its own indications and interpretation according to a specific set of data or operator selection (Koo & Li, 2016). In this study, intra-observer agreement was performed to assess the variation of measurements taken by two different operators. These were the only operators of interest in this study (but it is recognized that this does not necessarily represent the whole population of possible observers) which led us to choose the two-way mixed effect ICC at this stage with the absolute agreement only measured to be between both operators. The same principle applied to the intra-observer agreement and so this statistical test was applied to that study.(Hallgren, 2012)

The interpretation of ICC results has been defined by Koo & Li (2016) as follows

1. Value <0.5 is considered poor,
2. Value between 0.5 and 0.75 is considered to have a **moderate agreement**,
3. Value between 0.75 and 0.9 is considered to have a **good agreement**.
4. Results greater than 0.90 are considered to have an **excellent agreement**.

4.4 Results

The table below (Table 4.1) shows the detailed demographic details for both experiments. Females recruitment in both expirements was higher than males. An extra nerve was mapped in the inter-operator data due to the availability of an extra-participant at that time. All three situations of third molar categories were evident in both experiments

Category	***Inter-operator agreement***	***Intra-operator agreement***
Number of participants	9	8
Female: Males ratio	7:2	6:2
Numbers of mapped lingual nerve	11 (11.4%)	10 (10.4%)
Status of the third molar	Unerupted= 3 Partially erupted=4 Fully erupted=4	Unerupted=5 Partially erupted =3 Fully erupted=2

Table 4. 1 Detailed demographics of samples included in the inter- and intra-observer reliability.

4.5.1 Inter-observer agreement of mapping the lingual nerve :

The table below (4.2) shows all matching A points in very close alignments between different observers. At least two weeks between the two experiments was ensured. The maximum difference of 3 mm between the measurements was identified in the lingual nerve number 4.

Lingual nerve number	***A2 measurements (mm) (first operator)***	***A2 measurements (mm) (second operator)***
1	12	11
2	8	9
3	4	4
4	18	15
5	9	9
6	5	5
7	5	5
8	8	8
9	7	7
10	7	7
11	7	7

Table 4. 2 Point A measurements recorded by different observers. The red coded pair represents the one with the largest difference.

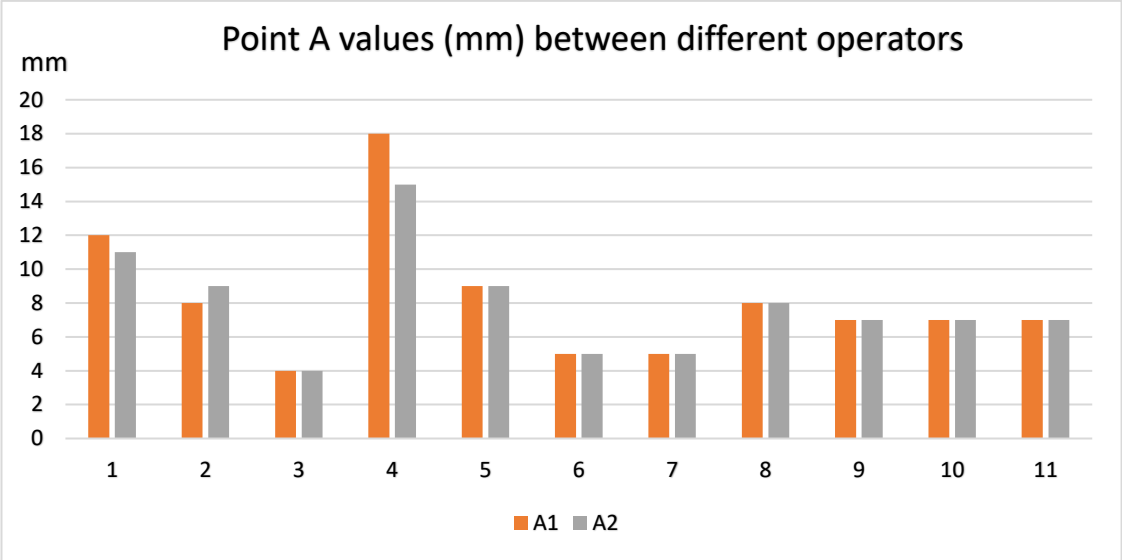

Figure 4. 1 Bar Chart illustrating the close measurements of point A between the two independent observers in 11 different lingual nerves

The table below (4.3) shows all matching B points in very close alignment between different observers. The maximum difference of 3 mm between the measurements was identified in the lingual nerve number 6.

Lingual nerve number	***B1 measurements (mm) (first operator)***	***B2 measurements (mm) (second operator)***
1	9	8
2	9	10
3	7	5
4	15	13
5	10	11
6	10	7
7	8	8
8	8	8
9	8	8
10	9	8
11	7	7

Table 4. 3 Point B measurements recorded by different operators. The red coded pairs represent the one with the largest difference.

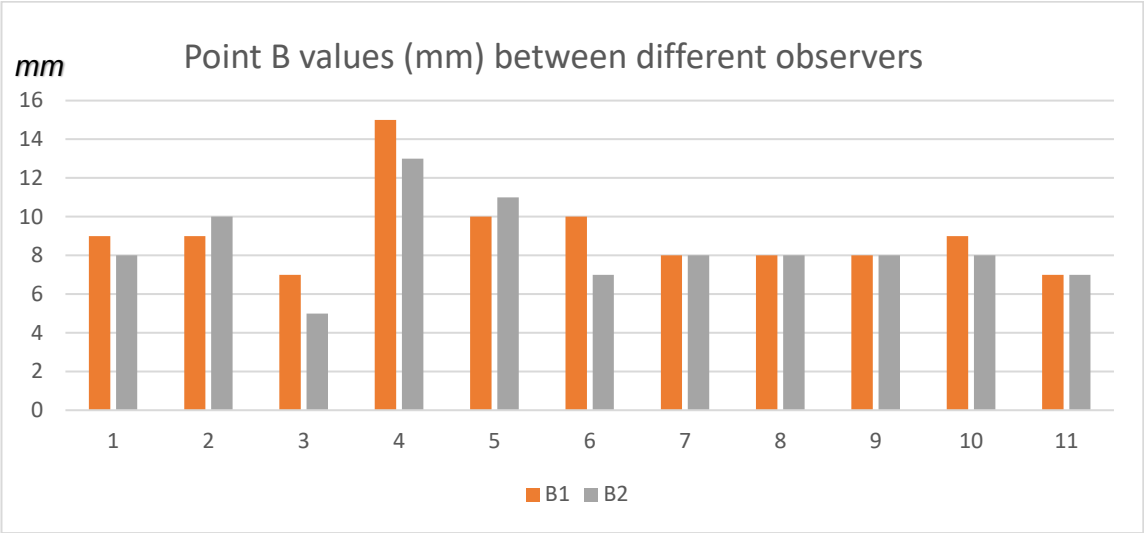

Figure 4. 2 Bar Chart illustrating the close measurements of point B between the two independent observers in 11 different lingual nerves.

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The table below (4.4) shows all matching C points. In two lingual nerves, the second operator was unable to identify this point. The measurements of the rest of the pairs were found be in close relation to each other with a maximum of 2 mm difference between the paired points.

Lingual nerve number	***C1 measurements (mm) (first observer)***	***C2 measurements (mm) (second observer)***
1	10	11
2	9	Unable to identify
3	5	4
4	15	Unable to identify
5	11	10
6	13	13
7	9	9
8	8	8
9	10	10
10	12	10
11	8	8

Table 4. 4 Point C measurements recorded by different observers. Note that the second operator was unable to identify point C in two nerves.

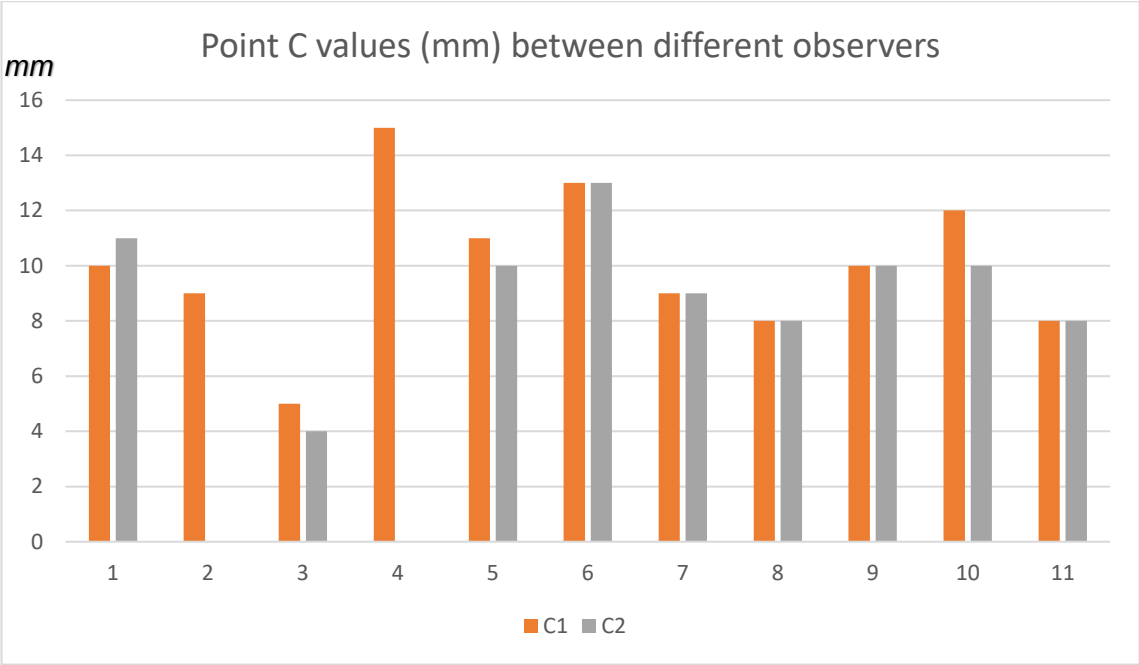

Figure 4. 3 Bar Chart illustrating the measurements of point C between the two independent observers in 11 different lingual nerves. The chart also shows missing values (by the second operator) in the 2nd and the 4th lingual nerves.

Intra-class correlation coefficient agreement between different operators of point A, B, C: Inter-operator agreement.

Table 4.5 below shows the agreement between the three paired points between different operators with the Confidence intervals and P values of each pair as follows:

Paired points	***95%Confidence interval (CI)***	***F Test with True value of ICC=0***	***ICC (two-way mixed effect)***
A1 versus A2	0.86-0.98	**P<0.001**	0.96
B1 versus B2	0.43-0.94	**P<0.001**	0.80
C1 versus C1	0.75-0.98	**P<0.001**	0.93

Table 4. 5 The value of inter-operator agreement using two-way mixed ICC of each paired points.

Although C point could not be identified by the second operator on two occasions, the ICC for the paired C points were higher than the paired B point. This was justified by the fact that the ICC was calculated only between the identified pairs that was relatively close in measurements than B points which had wider CI than point A and C. P value for all the results indicated that the measurements taken by both operators had true agreement.

4.5.2 Intra-operator agreement of mapping the lingual nerve:

The table below (4.6) shows the paired measurements of points A 10 lingual nerves were mapped in this study with least two weeks difference between the two experiments. The maximum differences of 2 mm between the paired measurements was identified.

Lingual nerve number	***Point A1 first measurements (mm)***	***Point A2 second measurements (mm)***
1	7	7
2	13	11
3	5	5
4	9	9
5	9	9
6	9	7
7	7	6
8	6	9
9	9	8
10	8	9

Table 4. 6 Detailed table of the measurements (mm) of point A in different experiments by the same operator. The red coded pairs are the ones with the maximum difference.

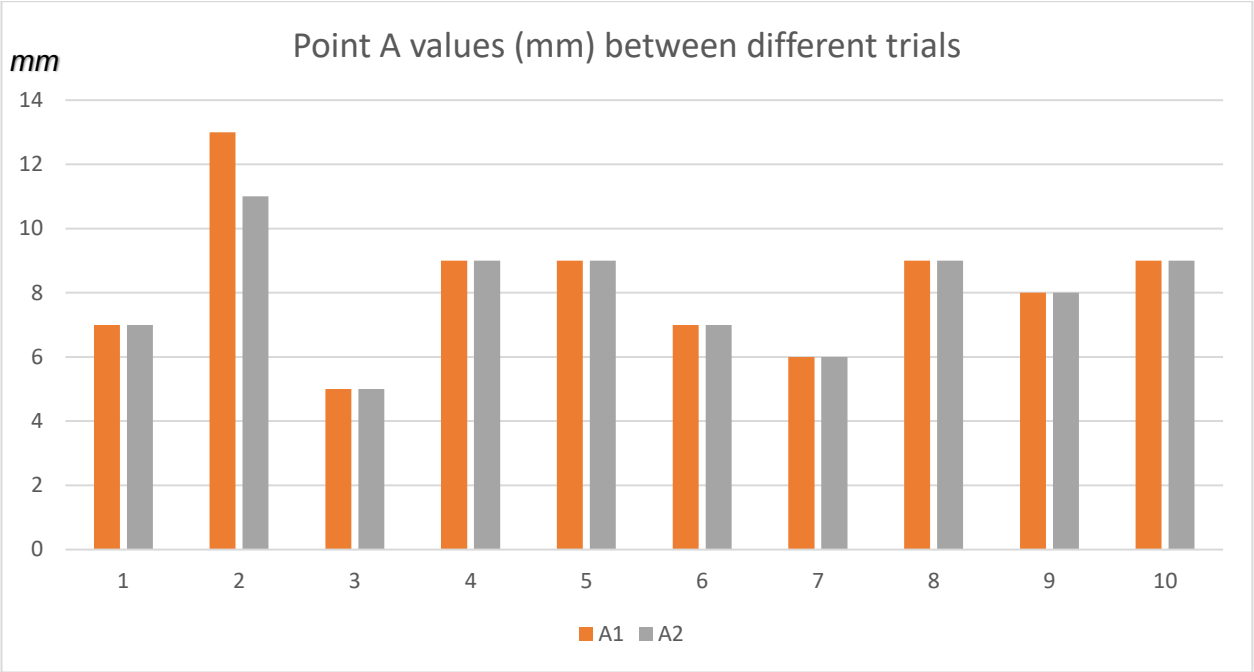

Figure 4. 4 Bar chart representing the measurements of point A in different trials by the same operator. A1 represents the first experiment in the first study. A2 represents the second trial at least two weeks later.

The table below (4.7) shows the measurements of paired B points in different experiments done by the same operator. This point was unable to be identified in both experiments in the lingual nerve number 2. The paired points showed close measurements across the sample with only 1 mm difference.

Lingual nerve number	***Point B1 first measurements (mm)***	***Point B2 second measurements (mm)***
1	9	8
2	Unable to identify	Unable to identify
3	5	6
4	11	12
5	8	8
6	7	7
7	10	9
8	10	10
9	9	10
10	9	9

Table 4. 7 The measurements (mm) of point B in different trials by the same operator. The red coded pair shows the points in which the operator was unable to identify point B in both experiments.

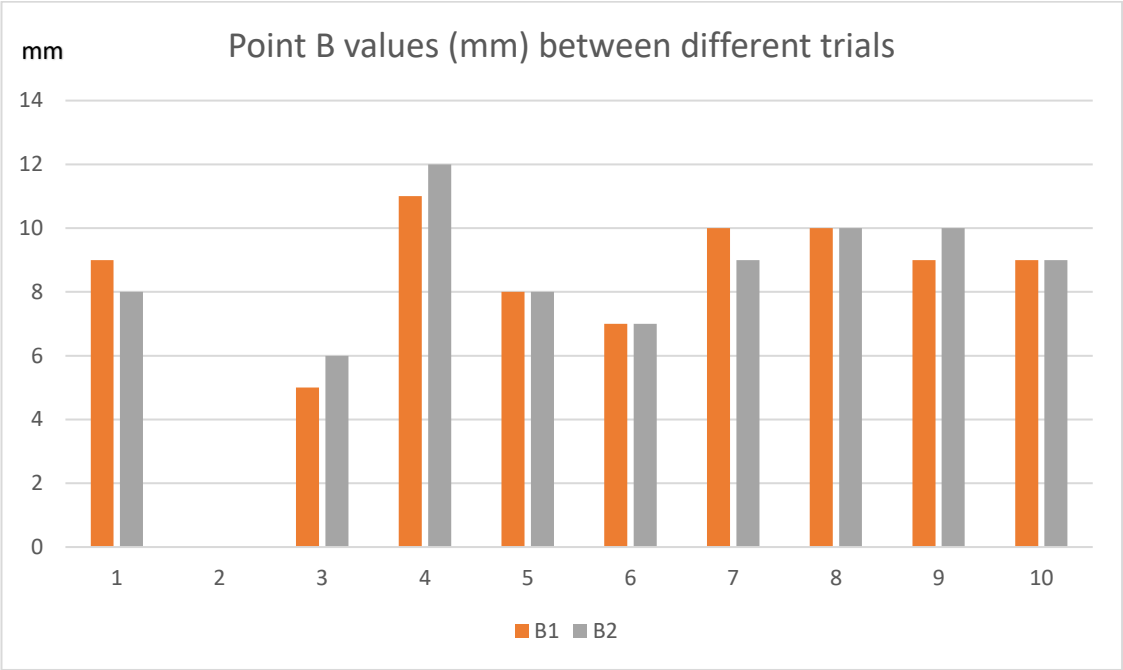

Chart 4. 1 Bar chart showing the value of point B in different trials by the same operator, (point B in the 2nd participant was unable to be detected in both trials). B1 represents the first experiment in the first study. B2 represents the second trial at least two weeks later

The table below (4.8) shows the measurements of paired C points taken by the same operator. In the second experiment, the operator was unable to identify the point in two lingual nerves. The paired points showed close measurements across the sample with only 1 mm difference

Lingual nerve number	***Point C1 first measurements (mm)***	***Point C2 second measurements (mm)***
1	12	12
2	13	13
3	8	8
4	13	Unable to identify
5	10	10
6	8	8
7	13	Unable to identify
8	13	12
9	10	9
10	10	9

Table 4. 8 Detailed table of the measurements (mm) of point C in different trials by the same operator. The red coded pairs shows that the operator was unable to identify points in the second experiment.

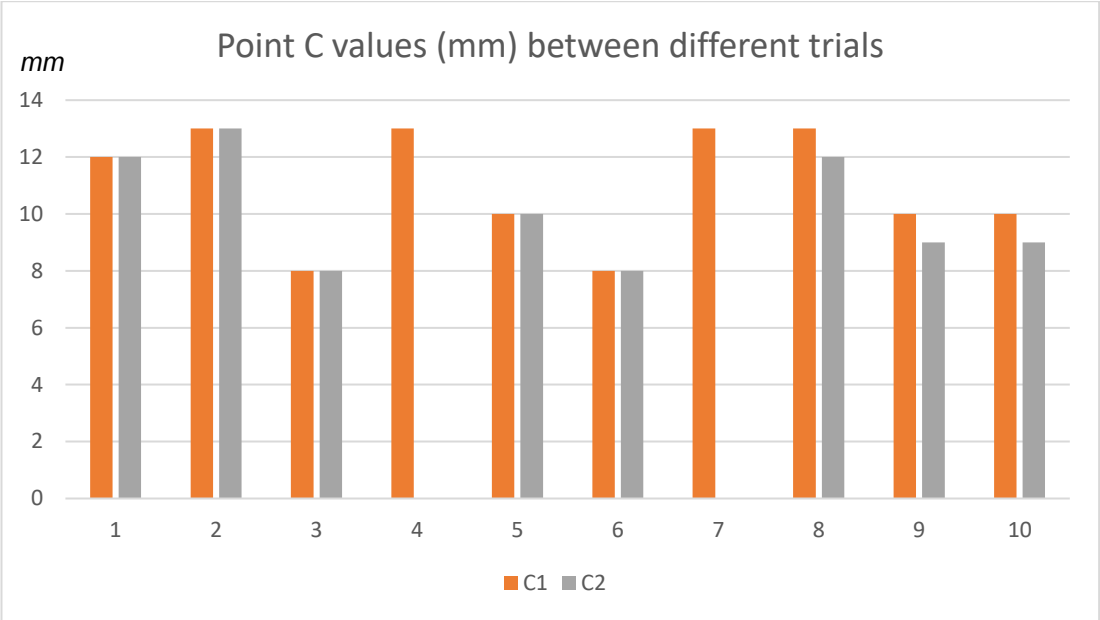

Figure 4. 5 Bar chart showing the value of point C in different trials by the same operator (Point C in the 4th and the 7th mapped nerve were unable to be identified in the second trial).

Intra-class correlation coefficient value for the same operator in two different trial: Intra-operator agreement*:*

The following table shows the agreement between the paired points using intra-class correlation coefficient.

Paired points	***95%Confidence interval (CI)***	***ICC (two-way mixed effect)***	***F Test with True value of ICC=0***
A1 versus A2	0.47-0.95	0.82	P<0.001
B1 versus B2	0.85-0.98	0.95	P<0.001
C1 versus C1	0.85-0.98	0.95	P<0.001

Table 4. 9 The value of intra-observer ICC with the limit of agreement of each paired points measured by the same operator.

Although C point was unable to be identified by the operator twice in the second experiment, the ICC for the paired C points were higher than the paired A points and equivalent to point B. This was justified by the fact that the ICC was calculated only between the identified pairs. P value for all the results showed that the measurements in both experiments had true agreement.

4.6 Discussion:

The results of both experiments have fulfilled the proposed aim in determining the level of agreement of this machine. Since this tool is still under investigation, testing both the internal validity and internal validity is of great importance. All the results of ICC in this experiment show good to excellent agreement in all the allocated points (above 0.8) in both inter and intra observer agreement.

Inter-observer agreement results were 0.96, 0.80, and 0.93 for A, B, C respectively. Intra-observer agreement results were 0.82, 0.95, 0.95 for point A, B, C respectively. Although the statistical interpretation of those results show good and excellent agreements, certain measurements in paired points were not able to be allocated. Calculating the agreement in these cases was performed by removing those paired points from the calculation as there were no numerical value of the unidentified point. This did reduce the number of pairs included but, did not show any statistical effect on the ICC value.

This way of analysing the data can affect the application of the results in real life. It can be postulated that if a certain observer or trial failed to identify the point of where the nerve is located using the EPT, then the clinical application of this tool could be negatively affected. In contrast, the discrepancy in those findings could also be observer or participant related and therefore, independent of the method itself. Improper moisture control at that point, participant positioning or the discrepancies in swiping the machine tip in the specific area between different operators would be an example of these factors.

The difference in measurnments of the mapped nerves at the three different points between both observers was considered to be within 2-3 millimetres. In clinical practice, this is not considered a significant difference between mapped nerves. This could be due to the variations between different observers in holding the EPT machine as well as visualising the measurements taken by the ruler, as this could be

affected by taking the measurements from a different angle. The ink diffusion could also have made slight changes of the measurements, especially at the very posterior point where there was increased risk of continuous pooling of the saliva. To reduce this risk in future studies, this could also be accompanied with a more stable measuring tool such as intra-oral calliper to reduce the chance of presentation bias between different observers and guarantee a fixed landmark.

Blinding of the results was ensured by collecting the data on a separate sheet. Despite the efforts to 'blind' the observers from previous results, this could not be 100% guaranteed, especially for the intra-observer agreement as the observer could potentially recall the previous position of the nerve. To minimise this, a minimum two weeks between the two trials was ensured

The idea of investigating whether an EPT is able to detect the lingual nerves that are situated in a vulnerable position or not, could not be investigated as the whole nerves in the sample of both inter- and intra-observer agreements were associated with cases where the nerve was positioned deeper in the tissue. It is unfortunate that this occurred as it would have been helpful to assess the ability of this technique to identify nerves in a vulnerable position. For future studies, increasing the sample size to accommodate more lingual nerves with a potentially superficial position would be recommended in order to identify the degree of agreement of EPT between different operators, or trials in identifying nerves with vulnerable positioning.

The good to excellent agreement in the overall results with a relatively small sample size cannot be generalised over the whole population. An increased sample size is needed to extrapolate the result more confidently to the general population. In addition, increasing the number of operators might increase the external validity but the recruitment of the sample can be more challenging due to inconvenience as well as increased demands on each participant.

4.7 Conclusion:

This experiment showed that the lingual nerve mapping technique using EPT, when able to detect the lingual nerve in situ, can be considered to give a reliable positional data of the lingual nerve when performed by the same observer on different occasions or by a different observer.

Chapter 5: Corroborating the clinical findings of the lingual nerve mapping using EPT with the actual position of the identified nerve using Magnetic reasonance imaging.

5.1 Introduction:

MRI has been used in clinical practice since the early 1980s. A marked development of both hardware and software technology has been witnessed since then. This continuous improvement of technology has facilitated better imaging quality, strength of magnetic field, scanning time and patient comfort. MRI is now the gold standard imaging technique in viewing internal brain pathology, extracranial nerves (Chen, 2007), as well as temporomandibular joint, salivary glands and soft tissue structures, which are poorly imaged by routine radiographic or ultrasonography techniques(Niraj *et al*, 2016).

The lingual nerve around the third molar region was studied using MRI for the first time by Miloro and his colleagues (Miloro et al, 1997). This group tried to identify the exact location of the lingual nerve on both the right and left sides in 10 healthy volunteers utilising high resolution MRI scan (HR-MRI) in both axial and coronal views. They found that 10% of the nerves (2 out of 20) were positioned above the lingual crest and 25% of nerves were in direct contact with the lingual plate from the coronal view. The nerve position was however studied at only one point - the middle of the third molar area (Miloro et al, 1997). In 2014, Cassette and colleagues used a stronger magnetic field MRI, 3 Tesla (3T), in detecting the largest division, the mandibular branch, of the trigeminal nerve. The course of the lingual nerve was described in this study in fine detail which suggested that the use of a stronger magnetic field, 3T, is beneficial in tracing the lingual nerve distribution in the lingual tissue (Cassetta *et al*, 2014).

The detailed physics of MRI is outside the scope of this thesis but a brief explanation of the technique follows:

MRI images are produced by the interaction between the magnetic field and the positively charged hydrogen protons in water and fat molecules of living cells. In the absence of a magnetic field these ions are randomly aligned. Once excited in a magnetic field the ions align parallel to the magnetic field (Currie *et al*, 2013; Sharma, 2009). When a radiofrequency current excitation is switched off, but a magnetic field remains, the protons tend to realign with the magnetic field. Simultaneously they emit a radio signal. The emitted energy from realigning protons is detected using antennae (coils) and can be used for making detailed images of body tissues. Differences in the energy emitted and the time taken for realignment between the scanned cells leads to a difference in relative image density between various structures (Currie et al, 2013).

Different imaging protocols and sequences can be utilised to view differing tissues, and with relevance to the current thesis, the segment of the nerve to trace its course in the tissue at specific points. Imaging the complicated structures surrounding those nerves, as well as their fine and small structure, still remains challenging whenever studying their course is undertaken (Cox et al, 2016; Miloro & Kolokythas, 2011). Improving fine detail visualisation can be altered by adopting certain imaging sequences and planes, trying different scanning protocols, and utilising specific sets of coils (Miloro & Kolokythas, 2011). Also a deep understanding of specific anatomical landmarks, and detailed anatomy of the studied region, is considered paramount for the ability to visualise nerves on MRI setting.

Fujii colleagues have also investigated the feasibility of using 3D Double-Echo Steady with water excitation sequence (3D-DESS WE) which is normally used in musculoskeletal imaging to view the branches of mandibular nerve (Fujii et al, 2015b). This sequence was first utilized in 2011 to view the facial nerve in the parotid gland, and was considered excellent for its ability to delineate the peripheral nerves and enhance their visibility as a high-intensity structure compared to the surrounding soft tissue (Qin *et al*, 2011).

Topography of the lingual nerve and the cross-sectional anatomy:

The proper understanding of the relation of this nerve to the surrounding structure is paramount to aid its identification. Knowing the anatomy in different sections of the lingual nerve is needed so the observer will be able to understand and outline the specific area of where to search for the nerve. As mentioned earlier, the lingual nerve leaves the skull as part of the posterior division of the mandibular nerve which exits the base of the skull via foramen ovale. One centimeter below the base of the skull, the lingual nerve branches off from the mandibular nerve and joins the Chorda tympani at a relatively acute angle. As it continues its way within the lingual mucosa of the mandible, it lies relatively superior to the mylohyoid muscle, but is not necessarily superior to its origin in the mandible. This can be explained by the funnel shape of the mylohyoid muscle, that runs obliquely in the floor of the mouth and is considered the main muscle involved in moving the floor of the mouth. After this, the lingual nerve then travels forward in the lingual mucosa covering the lingual aspect of the mandible, dips inferiorly and medially sandwiched between the hyoglossus muscle medially and mylohyoid muscle laterally. The submandibular duct can also cross over the lingual nerve in a relatively anterior position to the third molar.(Fazan *et al*, 2007; Sittitavornwong et al, 2017). The designed figure 5.1 shows the relation of the lingual nerve to the surrounding structures around the third molar area.

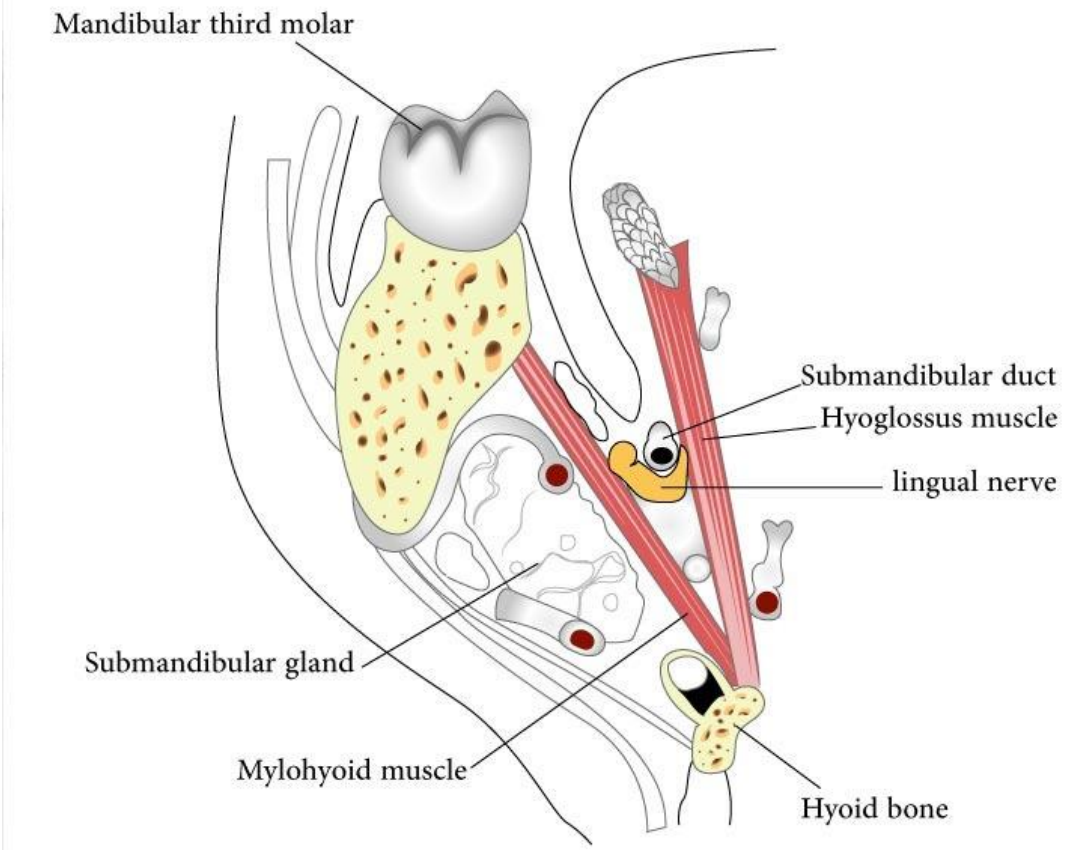

Figure 5. 1 Illustrating a cross section of the mandible at the third molar region which shows the lingual nerve and its relation to the surrounding muscles and other structures.

5.2 Aims and objectives:

The aim of this study was to:

1. Test the correlation in the position of the lingual nerves between these two methods in the same small sample of subjects.

2. Investigate whether the nerve position, as estimated by this method of the lingual nerve mapping, would corroborate results gained from the current gold standard imaging technique, MRI.

5.3 Methodology:

5.3.1 Ethical consideration:

This study was approved by the Ethics Committee of University of Liverpool after applying for an expedited review of a research project, in conjunction with the first and the second phase of this project A risk assessment form was also submitted with the research application and attached in the appendix. (Appendix1-3).

Reimbursement:
As in the first and second phases, a small reimbursement of no more than £10 per hour, to compensate for the inconvenience of travel and experimental time, was offered.

5.3.2 Study population and recruitment criteria:

Participants from the first and second phase of the study were asked to participate in this phase. The selection was aimed to target participants with variable nerve readings in the lingual tissue such as superficial,

low deep and moderate vertical nerve height. The detailed selection criteria are as follow:

A) Inclusion criteria:

Participants who already participated in the first and the second phase of the study. This was to corroborate their findings of EPT lingual nerve mapping with the identified nerve on MRI.

B) Exclusion criteria:

1. Participants with known claustrophobia. The MRI machine design resembles a closed cylindrical tube. The participants should stay still in the machine for about 20 minutes. Any participants with claustrophobia will not be able to tolerate these settings. From an ethical point of view, those participants were excluded as this can cause discomfort and irritation to the scanned participant.
2. Pregnant or breast feeding females.
3. Participants with metal objects or devices implanted in the body like pacemakers, heart valves and implants in the head and neck region. This can cause tissue overheating, discomfort and metal artefacts that would affect the image quality.

The participation was voluntary and participants were informed of the purpose as well as the technical side of the experiment through a detailed participant information leaflet. This leaflet is attached in the appendix section of the thesis (Appendix 9). A total of 7 participants has agreed to participate in this study. Only five were eligible to participate in the study (three females and two males). Figure 5.2 shows the recruitment chart and the flow of participation in this study.

Recruitment chart:

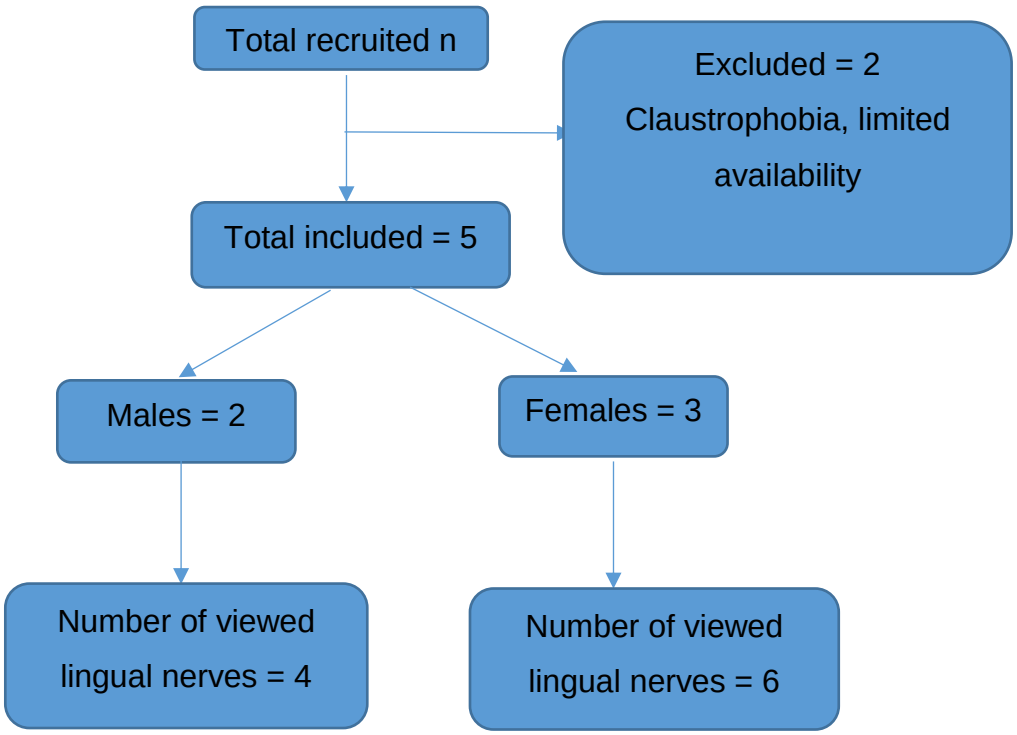

Figure 5. 2 Recruitment chart shows the flow of recruited participants in the study and the number of the identified lingual nerves.

5.3.3 Participant preparation:

All eligible participants were made aware that no metal clothing, jewellery or metal accessories should be worn on the day of the scans. Females were instructed to remove any hair metal clips or piercings as well as avoiding any makeup products or hair oils that could harbour any metal traces, which can overheat the tissue surface or cause skin irritation. All the above mentioned conditions were covered in standard set of guidelines that was followed in the MARIARC (Medicines & Agency, 2007). All participants were educated about the scanning process via an information leaflet, and a pre-scanning checklist to identify eligibility for the MRI scan was completed for every participant. These information sheets and MRI checklist are attached (Appendix 9, 10).

5.3.4 Sample size:

This study was conducted as a part of the feasibility study to correlate the nerve position between two different techniques but MRI scanning is not a routine pre-operative exam. One of the reasons for this is the high expense, as well as the limited availability of the machine in the clinical setting. By including 5 participants in the study, 10 lingual nerves were scanned. This was a total of 10% of the whole sample. Recruiting subjects for this study was limited due to participant availability and limited funding. Following the statistician advice, 5 participants, 10% of the original sample, were considered to be the minimum acceptable number in order to have data that can be analysed and detect any statistical significance.

5.3.5 Development of MRI Imaging protocol:

Pilot scanning:

The MRI machine utilised in the study was (SIEMENS MAGNETOM) Prisma with magnetic field strength of 3 Tesla. A healthy volunteer, outside of the study population was consented, and agreed to undertake this trial. The main purposes of this pilot scan were to:

1) Agree on a standard set of scanning sequences that could provide the most predictable view of the lingual nerve in the third molar region.
2) Identify any patient related factors that could affect the image quality such as tongue positioning.
3) Investigate the use of extra-oral head and neck coils and develop a new coil arrangement to maximise resolution of the resultant image.

Following consideration of this initial scan, the final scanning of the lingual nerve was composed of different sequences as follows:

1. Coronal T1-weighted STIR sequence of 2 mm slice thickness. (t1_tirm_cor_320)
2. Coronal T2-weighted DE 3D WE sequence of 0.6 mm slice thickness. (t2_de3d_we_iso_0.6_ISO_COR)
3. Axial T2- weighted DE 3D WE sequence of 0.7 mm slice thickness. (t2_de3d_we_iso_0.7_TRA)
4. Axial T2-weighted DE 3D WE sequence of 0.6 mm slice thickness.(t2_de3d_we_iso_0.6_TRA)

Justification of the developed scanning sequence

As part of the routine scanning protocol, initial acquisition of short T1 inversion recovery (STIR) was used to view any incidental findings of any gross pathology in the head and neck region. This sequence is known for nonselective fat suppression with high affinity to water molecules which compose the majority of pathological lesions. According to updated literature, the most favourable scanning sequence for viewing extra-cranial nerves was T2 3D DESS WE. This was adopted in both axial and coronal views (Fujii *et al*, 2015a) (Qin et al, 2011). The resolution of this sequence was determined by the size of voxels that can be pre-set. An axial view of T2 3D DESS WE sequence was tried in both 0.6 mm and 0.7 mm but more resolution was obtained from the 0.6 mm slice thickness, which was subsequently adopted in the scanning protocol for the study in both axial and coronal views. However, the main drawback of reducing the slice thickness was increasing the scanning time to almost double, which would play a role in increasing motion artefact (Cassetta et al, 2014). The 3D imaging of the scanned area by the MRI can be presented in different views or reconstructed images from the raw data. In the current study, each view was utilised from its raw data scans rather than being reconstructed from different views.

The table below (5.1) shows the detailed scanning parameters of each sequence utilised in this study that was performed in the MARIARC:

Scanning parameter /Scanning sequence	***T2 DESS COR 0.6 mm***	***T2 DESS TRA 0.7 mm***	***T2 DESS TRA 0.6 mm***	***T1 STIR COR 2 mm***
TR	13.95 ms	13.59 ms	13.95 ms	565 0.0 ms
TE	4.9 ms	4.91 ms	4.9 ms	20 ms
slice thickness	0.6 mm	0.7 mm	0.6 mm	2 mm
RESOLUTION	0.6 X 0.6 X 0.6 mm	0.7 X 0.7 X 0.7 mm	0.6 X 0.6 X 0.6 mm	0.6 X 0.6 X 2.0 mm
FOV	230 X 186.9 mm	230 X 186.9 mm	230 X 186.9 mm	230 X 186. 9 mm
NEX	1	1	1	1
MATRIX	384X 293	320X 260	384X 293	
FLIP ANGLE	25 deg	25 deg	25 deg	150 deg
SCAN TIME	09:20 min	05:34 min	09:20 min	4:30 min
NUMBER OF SLICES	160	160	160	32
PHASE	R>L	R>L	R>L	R>L

Table 5.1 Detailed parameters of each sequence involved in the MRI scanning protocol. TR = repetition time, TE= echo time, FOV= field of view, NEX= number of excitation.

The lingual nerve was identified in both the T1, and T2 weighted scans in close proximity to the cranial base after exiting the foramen ovale. The close radiopacity of different structures existing near the lingual nerve made the identification of the nerve more challenging. The position of the tongue also challenged identifying the position of the lingual nerve. As the subjects were scanned in a relatively supine position with the mouth closed, the resting position of the tongue was in intimate contact with the lingual mucosa. Obviously in the coronal section, this would show the third molar and its thin lingual mucosa in a continuity with the tissue of the tongue which rendered it hard to separate, or identify, the borders. In order to overcome this obstacle the creation of a separator, to delineate the borders of the tongue, was investigated to limit the overlapping with the lingual mucosa of the studied area. Although this was hard to achieve, participants were also instructed to prevent the voluntary movement of the tongue and reducing the swallowing whenever the acquisition was active as this could cause motion artifacts.

Development of intra-oral separator:

In order to reduce the density of the structure at the lingual side and create a space between the tongue and the lingual mucosa of the third molar, a separator between the tongue and the lingual surface of the mandible around the third molar area was considered. Custom-made mouth guards with extension into the lingual pouches were constructed to separate the lingual mucosa and the lateral border of the tongue.

Investigation of the safety of intra-oral separator material:

The material used to fabricate the intra-oral tongue separator was tested in the MRI machine to check for the feasibility of its use. Three sample materials considered for fabrication of intra-oral separator were investigated for their safety and the amount of artifact emitted from them whilst scanned in the MRI machine. The main criteria for their

use was the absence of metal particles in their composition as well as being able to be placed in the mouth with no irritation or discomfort. Revising the ingredient leaflet as well consulting an expert dental technician regarding the composition of each material was done prior to selection. The Figure below (5.3) shows the selected materials:

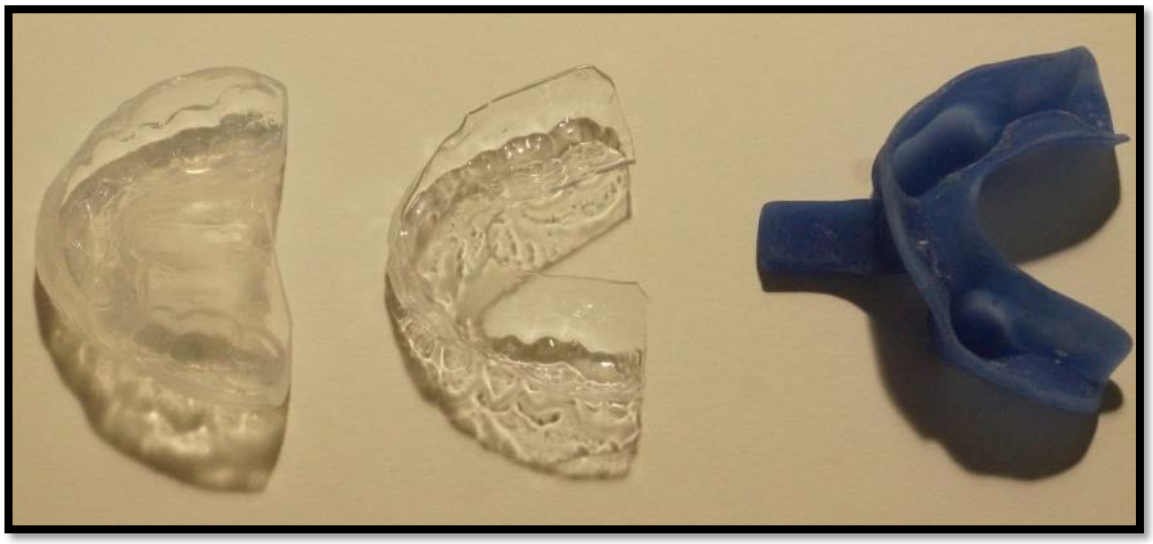

Figure 5. 3 Showing from left to right, 4mm thickness high impact thermoplastic polystyrene sheet, 2mm thickness high impact polystyrene sheet, light cure acrylic for special tray fabrication.

An MRI expert radiographer investigated the materials in the MRI machine by running the same sequences developed in the pilot scanning. The three samples were placed in a plastic tub filled with water. Since the MRI machine targets water molecules that is mainly composed of hydrogen protons, this was considered to be a medium that resembled the oral environment (as almost 70% of the body is composed of water). The main reason for this stage was to investigate the level of noise being emitted by each material. Image analysis showed some artifact from the plastic tub but the light cure acrylic showed more artifacts compared to the high impact acrylic. Based on the findings of the screening scan, and the composition of the materials, the high impact acrylic was prefered to fabricate the intra-oral separator. The 4mm thickness was selected to guarantee more separation between the tongue and the lingual tissue.

Fabrication of intra-oral separator:

In order to perform a custom-made lingual separator, a primary impression of that area was taken using alginate impression. Following immediate cast pouring, a custom tray impression was fabricated with sufficient extension to support impression material in the posterior lingual area of both right and left sides. The master impression was then taken by border moulding of the lingual area with green stick followed by an alginate wash. The impression then was poured using dental stone and the master cast was trimmed in order to have an optimum fit in the vacuum machine (Druformat SQ). Thermoplastic high impact polystyrene 4 mm thickness sheet was placed and the vacuum machine was and heated then pressed to the cast and left until it cooled down. The cast then was removed from the vacuum machine and the excess of the material was trimmed. The material was carved to be only adapted to teeth apart from the lingual extension in the third molar region in which the separator was kept to the full depth of the lingual sulcus. The margins were ensured to be smooth prior to fitting in the participant's mouth to prevent irritation. Figure 5.4 (A&B) show both frontal and intra-oral view of the intra-oral separator and its extension to the lingual tissue.

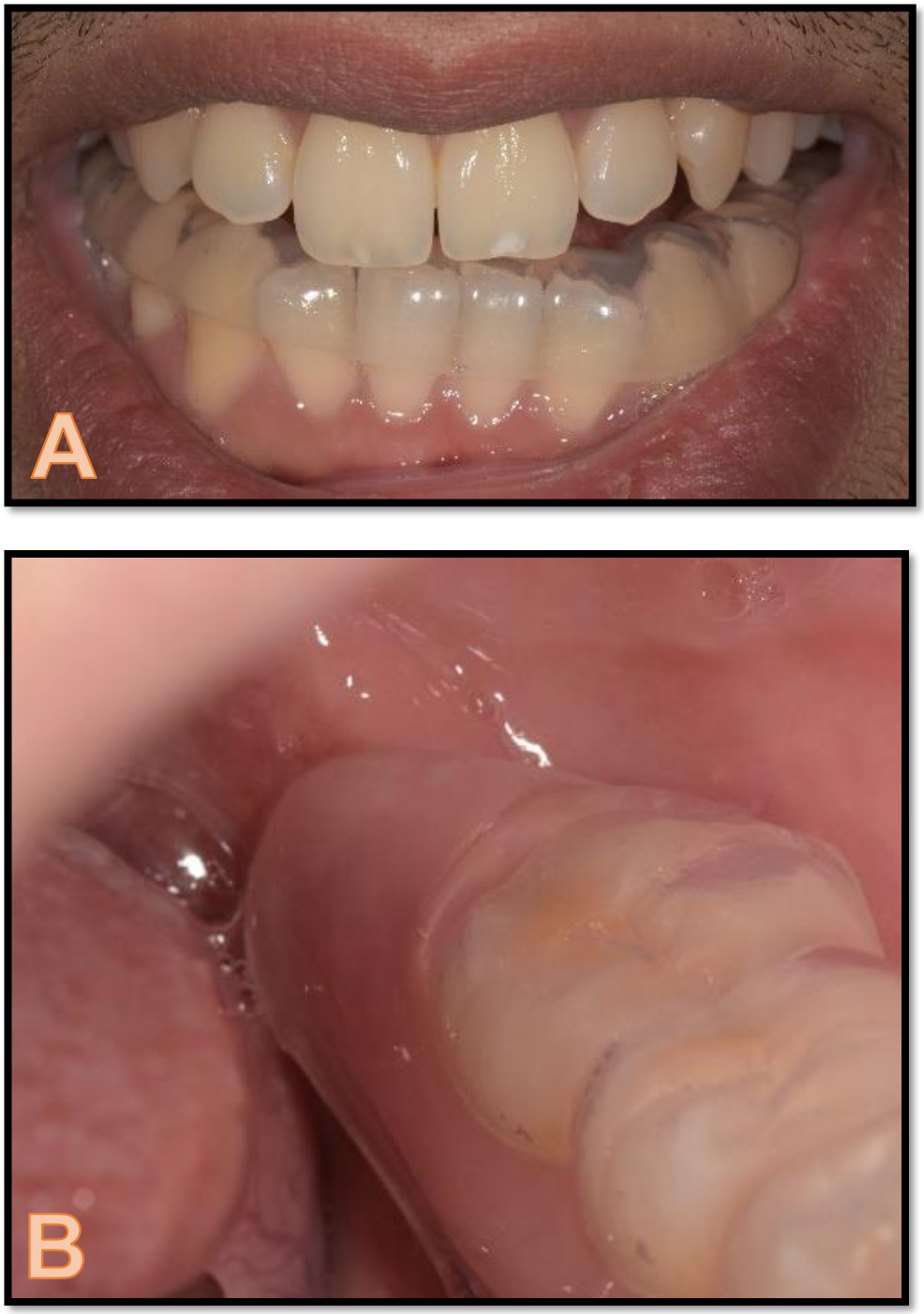

Figure 5. 4 Frontal photograph A showing the fitting of intra-oral separator. Intra-oral photograph B showing the extension of the separator in the left side third molar and retromolar pad area.

Extra-oral Coils arrangement:

In order to improve the quality of the MRI images, the signal emitted from the scanned tissue should be higher than the noise. Increasing the signal to noise ratio, will increase the resolution of the scanned area. Extra-oral head and neck coils are usually adapted whenever that area is going to be scanned. The use of these coils close to the scanned area helps to pick up signals from the tissue more efficiently, thus providing more information. Different sets of extra-oral coils are available in the market and a trial of a specific configuration was adopted in this study in order to enhance image quality and resolution of the scanned intra-oral area. Both a 64 channel head and neck coil and flex small 4 channel coil were beneficial in inducing better image quality, and so this was adopted in the current scanning protocol. Figure 5.5 (A&B) shows the extra-oral coils and their arrangements as follows.

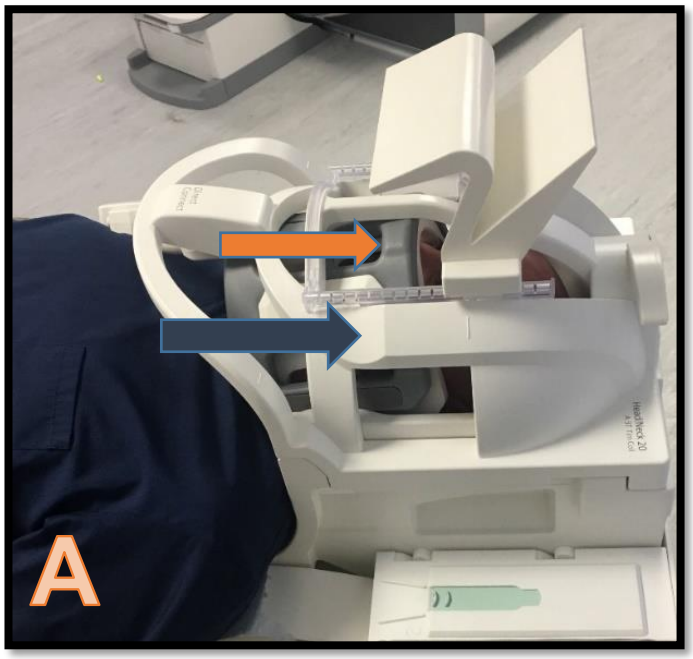

Figure 5. 5 Extra-oral coils arrangements adopted in the study. The small flex coil wraps around the lower half of the face where the lingual nerve is studied (yellow arrow in both photographs A&B). The 64 head and neck coil in picture A is then placed to cover a larger area of the head and neck (blue arrow).

Image analysis and data collection:

The MRI scans were viewed using SyngoVia software on a 24-inch workstation. One examiner was involved in viewing the lingual nerve, student investigator (SA), who had trained in viewing the lingual nerve on MRI scans by one of the consultants with an interest in the MRI field. The lingual nerve was located as it exited from the base of the skull at the foramen ovale and its course followed until it got to the lingual surface of the mandible, adjacent to the third molar. This was done alternating between axial view and coronal views. Coronal views of 0.6 mm slice thickness 3D DESS WE were used for taking measurements of the lingual nerve. A standardised data collection protocol was used:

- Define the distal of the second molar tooth as the initial landmark.
- Measurement of point C reading from top of the distal interproximal gingival soft tissue.
- In erupted third molars point B was taken as the midpoint of the crown the tooth. In unerupted molars it was taken as the midpoint between points A and C.
- Point C was derived from the clinical measurement between A-C taken during nerve mapping. This distance in mm was divided by 0.6 (slice thickness) to give number of slices movement from point C to arrive at point A

5.3.6 Statistical analysis:

Data evaluation was performed using statistical analysis software (SPSS® IBM Corporation Statistics). The nature of the data was categorized as continuous measurements.
Reproducibility and agreement between the two different methods in identifying the lingual nerve height in the lingual tissue was conducted using two-way mixed Inter Class Correlation with testing of the absolute agreement. This was justified for the following reasons:
1) The test is concerned in measuring the agreement between these two methods only, irrespective of other methods.
2) These two methods are measuring the same participants using the same reference points to get a continous measurements.
3) The absolute agreement is performed to identify the extent to which each measurements of the MRI equals the corresponding measrnment of the EPT.(Hallgren, 2012) (Koo & Li, 2016).

5.4 Results:

Selected cases with an identified lingual nerve at different heights:

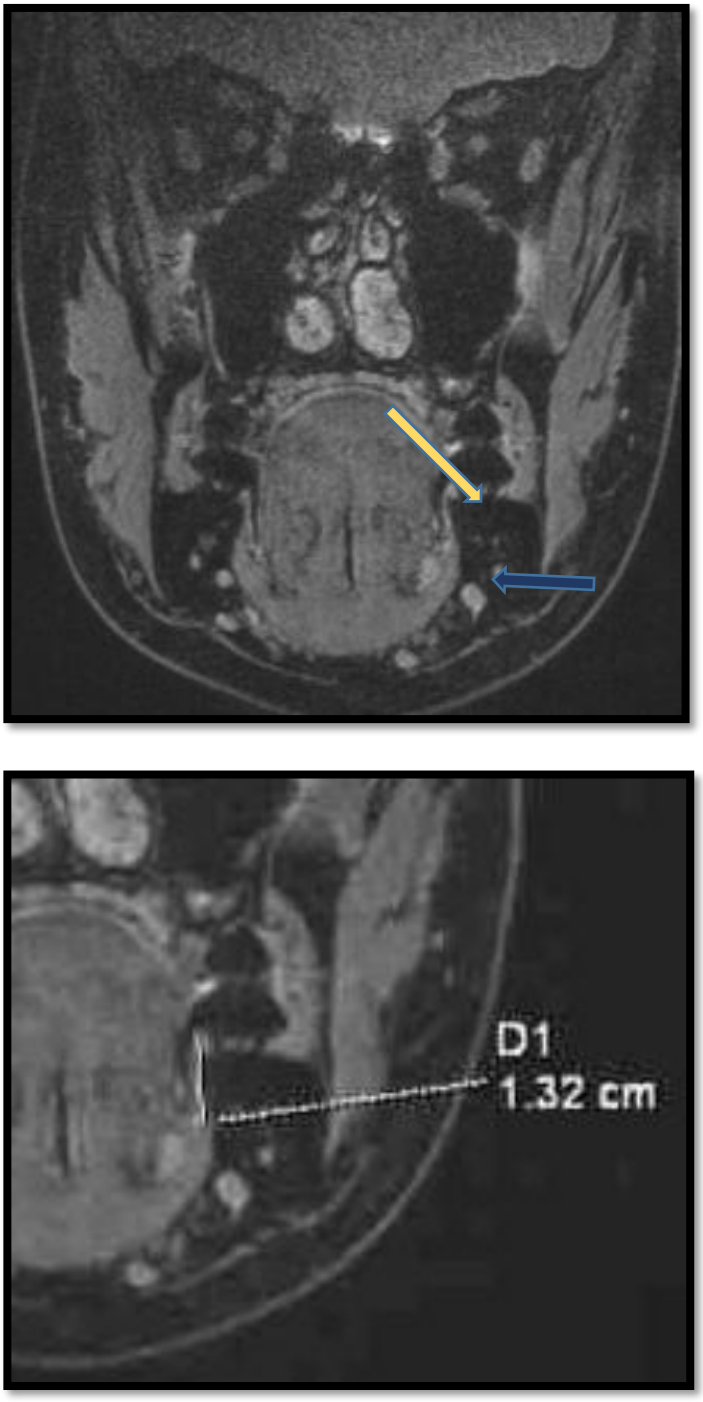

Figure 5. 6 MRI coronal view of T2 3D-DESS WE at the level of an erupted third molar region, (point B) notice the intra-oral separator (yellow arrows) and the identified lingual nerve on the right side at point B (blue arrow) this was measured to be 1.32 cm (13 MM).

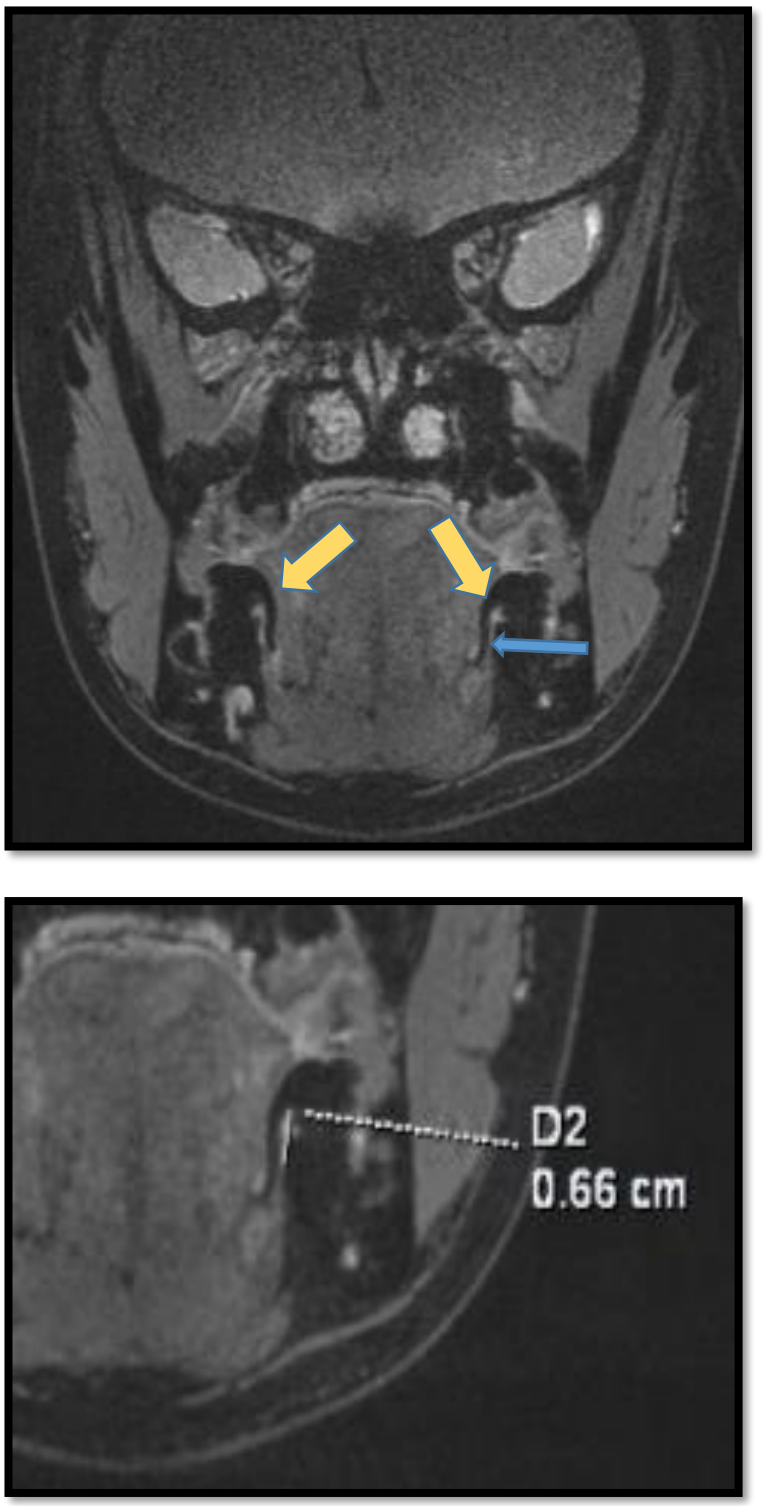

Figure 5. 7 MRI coronal view of T2 3D-DESS WE at the level of an erupted third molar region,(point B) notice the intra-oral separator(yellow arrow) and the identified lingual nerve on the right side at point B this was measured to be 0.66 cm (6.6 mm).

Demographic data of the study:

The table below (5.2) the demographics of the study sample which was recruited from the original sample (10% of 50 participants). Male to female proportion was representative of the original study sample with the mean age of 25 years old. Both right and left lingual nerves of each participant was scanned and identified.

Demographic Data	***Number (percentage)***
Males	2 (40%)
Females	3 (60%)
Total participants	5 (100%)
Age range	21 to 30 y
Mean	25 y
Total nerve scanned	10
Total identified nerves	10

Table 5.2 Demographics of the study population.

Findings of MRI positional data of point A, B and C and their corresponding results from the first study using EPT:

Point A participants	*A1 Value on MRI*	*A2 Value on EPT*
Participant 1		
A right	6 mm	5 mm
A left	Unable to locate	5 mm
Participant 2		
A right	12 mm	10 mm
A left	10 mm	10 mm
Participant 3		
A right	6 mm	5 mm
A left	5 mm	4 mm
Participant 4		
A right	8 mm	9 mm
A left	8 mm	8 mm
Participant 5		
A right	9 mm	9 mm
A left	Unable to locate	18 mm
Mean value:	8 mm	8.2 mm
Standard deviation:	2.3 mm	4.1 mm

Table 5.3 Compares point A measurements in the five participants on both right and left sides. A1 and A2 were close in measurements across the sample. A1 was unable to be detected in 2 lingual nerves (A1 = MRI, A2 = EPT)

Point B Participants	***B1 Value on MRI***	***B2 Value on EPT***
Participant 1		
B right	8 mm	8 mm
B left	5-7 mm large cross section of the nerve	7 mm
Participant 2		
B right	9.8 mm	10 mm
B left	15 mm	15 mm
Participant 3		
B right	6 mm	5 mm
B left	5.6 mm	7 mm
Participant 4		
B right	12 mm	10 mm
B left	Unable to locate	10 mm
Participant 5		
B right	Unable to locate	11 mm
B left	10 mm	15 mm
Mean value:	8.90 mm	9.8 mm
Standard deviation:	3.46 mm	3.2 mm

Table 5.4 Compares point B measurements in the five participants on both right and left sides. Point B1 and B2 were close in measurements across the sample. Only in one sample, 5 mm difference was identified between B1 and B2 in the 5th participant B1 was unable to be detected in 2 lingual nerves using MRI. (B1 = MRI, B2 = EPT)

Point C Participants	***Value on MRI***	***Value on EPT***
Participant 1		
C right	11 mm	9 mm
C left	8 mm	8 mm
Participant 2		
C right	9.7 mm	6 mm
C left	14 mm	15 mm
Participant 3		
C right	8 mm	8 mm
C left	6 mm	5 mm
Participant 4		
C right	10 mm	13 mm
C left	12 mm	12 mm
Participant 5		
C right	Unable to locate	13 mm
C left	10 mm	15 mm
Mean value:	9.8 mm	10.4 mm
Standard deviation:	2.3 mm	3.6 mm

Table 5. 5 Compares point C measurements in the five participants on both right and left sides. In two points, the difference between C1 and C2 was more than 4 mm. C1 was unable to be detected in 1 lingual nerves using MRI. (C1 = MRI, C2 = EPT)

Paired Points	***95%Confidence intervals***	***F test with true value of ICC=0***	***ICC Two-way mixed effect***
A1 vsA2	0.54-0.98	P<0.0001	0.92
B1 vs B2	0.39-0.96	P<0.0001	0.82
C1 vs C2	0.06-0.92	P<0.003	0.68

Table 5. 6 The level of agreement with 95% CI of the paired points between MRI, and EPT tests. (A1, B1, C1= measurements in MRI, A2 B2, C2= measurements in EPT)

The above table shows the results of ICC in all paired points. Point C appears to have the least agreement (ICC= 0.68) with wide CI. Point A and B have shown higher agreement with narrower CI. P value for all the results showed that the measurements in both techniques was as a result of true agreement.

5.4 Discussion:

This study was conducted to investigate whether clinical readings of the lingual nerve position obtained by EPT measurement would compare with positional data of the lingual nerve near the mandibular third molar as seen on MRI. MRI is a well-established imaging technique to view extra-cranial nerve tissues, with no risk of ionizing radiation. Although in this study it was considered to be the most convenient technique for visualising the lingual nerve, it is recognised as a reference standard in this study. This is justified by the fact of not being able to localise the nerve in all the sections of the MRI scans in this study. Concisely, in order to be able to identify the true anatomical position of the lingual nerve, an incision of the overlying mucosal tissue around the studied area is to be performed. This was carried out in one clinical study as previously described in Kieselbach 1985 which were not deemed ethical in healthy research participants and the protocol was considered outdated.

Due to cost issues and availability of both participants and the scanner time, the sample size of this study was limited to five participants, 2 males and 3 females. The recruitment in this study aimed to have participants with different nerve heights, previously mapped with EPT, in order to be able to identify whether the newly developed EPT is reliable in identifying the lingual nerve with different heights in the third molar area. Both right and left side lingual nerves were scanned to provide a sample of just over 10% of the total lingual nerves of the original sample.

We developed a novel protocol to overcome certain factors that prevented the visualisation of the lingual nerve in the pilot scanning. An intra-oral tongue separator and extra-oral coil arrangements have contributed to the successful delineation of the lingual nerve from the adjacent structures, as well as picking up more signals from the scanned tissue to provide a better resolution quality. However, the Intra-oral mouth separator

increased the pooling of saliva which, as a result, could have led to repetitive swallowing this was thought to contribute in increasing the motion of the patient which would lead to motion artefact.

3D-double echo steady state with water excitation 3D DESS WE has been reported to give a better definition of extra-cranial nerve branches (Fujii et al, 2015b; Qin et al, 2011). Although this scanning protocol was adopted, the lingual nerve was still difficult to visualise along its entire length. It could be argued that the nature of the nerve, in the study area, can harbour a small diameter, an abrupt changes in directions as well as reduced fat distribution which could make it difficult to view the nerve by the MRI as it advances towards the tongue in the pterygomandibular space (Tan et al, 2014). This required a protocol whereby the nerve was identified as it exited the skull base and followed along its course through multiple images slices. At some points drop out of signal would be present but picked up again on the following image slice

.

Unlike radiographic images, MRI permits the different soft tissues to be relatively distinguishable. This allowed us to make the reference point of each measurement of the lingual nerve height in the lingual tissue, the same as the clinical reference point (that had been used in the clinical mapping of lingual nerve using EPT). In each scanned nerve, a standardised protocol was followed to view the points A,B,C in coronal reconstructed slices to reproduce the clinical situation and reduce the risk of presentation bias. Scans were analysed by a single observer who had trained in viewing the lingual nerve in MRI scan by understanding the anatomy of the lingual nerve in relation to complex structures in the third molar region.

Unidentified points across the viewed lingual nerves were thought to be related to either motion artifact, which was evident in the 4th and the 5th participants or the position of the nerve in close relation to either submandibular gland duct or other structures of similar density.

Whenever the nerve was identified in both techniques, some points showed a difference of 3-5 mm between the paired points. This was suggested to be either multiple branches of the lingual nerve or motion artefact

The analysed data using ICC showed good to excellent level of agreement between the two different methods. Interpreting those results should be taken carefully as the pairs with the missing point were removed from the analysis which has contributed to reduced sample size. Point C was considered the point in which both EPT and MRI had least agreement (ICC= 0.6) while both Points A and B showed high levels of agreement of 0.92 and 0.82 respectively.

The available literature regarding the identification of the course of the lingual nerve is relatively limited. This is due to either studying the lingual nerve in different areas compared to this study i.e. more superior in the base of the skull (Fujii et al, 2015b) or to detect the usefulness of magnetic resonance in the management of neuropathy (Cox et al, 2016). In 1997, Miloro and colleagues performed their research in the same area as the current study. They studied the mean vertical and horizontal positions of the lingual nerve in relation to the alveolar crest in the third molar region and they found that the mean vertical distance in 20 lingual nerves was 2.75mm (range 1.52 - 4.61). It is important to mention however that Miloro et al, used the alveolar crest as the reference point. This is almost 2.5-3 mm deeper than the overlying soft tissue reference point in this study, which correlates well with the depth of the crevicular sulcus and the biological width .

Future recommendation

Improvements of scanning parameters are suggested for future research purposes by recommending the use of ultra-high strength of magnetic field 7 tesla (Hess, 2011). It is important to understand that the limited availability, high financial burden and the high susceptibility to other potential artifacts can be some of the drawbacks in the use of these high magnetic field machines, especially if it is adopted in the oral environment - which could have more metal particles implanted in the tissue.

Further improvement can also be considered in future scans by exploiting intra-oral coils that will increase the resolution of the images which helps in picking signals back from the fine structures (Ludwig *et al*, 2016). The main drawback of these coils however is the limited availability as well as the increased cost.

Although the idea of detecting the levels of sensitivity and specificity of EPT is of importance, the small sample size in this study could leave any statistically significant results to be highly misinterpreted. For future investigations an increased sample size is recommended which will give strength for the results to have more clinically significant results. Analysing the data by 'blinded' observers who have not contributed to the clinical EPT mapping of the lingual nerve should be considered to prevent bias.

5.5 Conclusion:

Within the limitation of this study, the agreement between MRI & EPT suggests the potential benefits of EPT as a tool in mapping the *in-situ* position of the lingual nerve.

Chapter 6: Conclusion in relation to clinical practice and recommendations for further research:

This project was designed to investigate the use of a proprietary EPT device to stimulate the lingual nerve and identify its *in-situ* position. To achieve the main aims and objectives of this project, a cross-sectional descriptive pilot study was designed and conducted in three different studies. The recruited population was selected from healthy participants most of which were dental students. In the first study, the concept of stimulating the lingual nerve by an electrical pulse from the EPT was investigated. This part was designed to ascertain that this machine is able to activate the lingual nerve tissue which was hypothesized to be the actual position of the nerve. Participants could not only detect a distinct projected sensation felt in the tongue on stimulation over the nerve, but also could distinguish between sensations on the mucosa alone verses projected sensations of nerve stimulation. This positive response from the participants with minimal adverse events was considered as a positive proof of concept.

Amongst the reported literature that investigated the position of the lingual nerve, we believe this study to be the only study that has investigated the *in-situ* location of the nerve using an electrical stimulus. The designated methodology was provided accordingly to replicate the clinical situation utilising the EPT machine which is readily available in the dental setting.

The second part of the first study expanded the sample size to investigate the positional data of the lingual nerve via mapping. The findings of this study were shown to be within the broad range of measurements of the reported literature.

In the second study, the reliability of the tool was investigated. Excellent inter-observer and intra-observer agreement in the lingual nerve measurements were found in this study, albeit with a small sample size.

In the third study, the results of mapping the lingual nerve with the EPT device were compared with the position of the lingual nerve as seen on MRI. Although the available literature has reported that identification of the lingual nerve using MRI was relatively straight forward, in this study we had to adjust the participant's tongue position, incorporating standard set of coils and understand the anatomy of the studied area more closely in order to be able to identify the lingual nerve in the third molar region. Within the limited sample size, we were able to conclude that the agreement in measurements of nerve position between these two different techniques was good.

Taken together these studies suggest that identifying the position of the lingual nerve *in-situ* in healthy volunteers with electrical stimulation using a simple proprietary EPT device is feasible, safe and practically achievable.

Refinement of electric stimulation device

Throughout the experiments as part of developing a technique, certain issues were raised with the machine handling and the design of the EPT. The drawback and the suggested modifications have been discussed in more detail in the preceding chapters but brief concluding points are as follow: a more rounded tip of the EPT device would have a less irritating surface and more easily adapt to the soft tissue, thereby enhancing comfort during nerve mapping. This may also reduce potential problems with suboptimal angulation of a flat tipped probe during activation of the nerve. The device tip could also be elongated and made thinner to reduce the amount of tongue retraction necessary during mapping. As part of activating of the tip of the machine, a beeping light was the indicative mark on the activity of the machine. This is available on the side of the machine and not quiet noticeable. To improve on the awareness of the activation status of the machine, a beeping sound could be considered to be more effective in order to identify if the machine is still producing an electrical stimulus.

Refinement of measurement technique

During this study a small intra-oral ruler was used to measure the distance from the soft tissue landmark to the position of the subject's perception of a projected sensation during stimulation. This is subject to inaccuracy due to changes in angulation between the observer's view of the ruler and the soft tissues. Utilising a stable measurement tool such as blunt tip callipers may facilitate more accurate measurements.

Recommendations for further research and other applications of EPT:

As the initial results from this pilot study has shown to have positive use of EPT in identifying the *in-situ* position of the lingual nerve, this study could be expanded to greater sample sizes allowing statistical analysis of population subgroups, for example, gender, age, race as well as gaining more statistical power.

This technique may be transferable to patient groups. Retrospective analyses of patients with the lingual nerve damage following surgery could be used to directly correlate nerve position with this unfavourable surgical outcome. Direct evidence of this nature would then underpin the use of this technique as a preoperative risk assessment tool.

A similar technique could also be adapted for the localization of other nerves, for instance, the distribution of the mental nerve prior to procedures such as mandibular vestibuloplasty or alveoloplasty. In this instance, the procedure would be the same but the nerve stimulator will be moved in vertical lines along the mucosal surface of the lip until a projected feeling is experienced and then marked.

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Appendices

Appendix 1 Application for ethical approval for the lingual nerve mapping and MRI scanning

APPLICATION FOR APPROVAL OF A PROJECT INVOLVING HUMAN PARTICIPANTS, HUMAN DATA, OR HUMAN MATERIAL

NOTES

1) This application form is to be used by researchers seeking research ethics approval from the University, as per the University's Policy on Research Ethics involving Human Participation. If an application qualifies for expedited review (Section C) it may be reviewed at Level 2, by your School or Institute's research ethics process.

2) Applications to the University Research Ethics Committees must normally include an **application form, participant information sheet and consent form** (all templates available online), along with any other relevant information, and should be submitted by email to the relevant contact listed at http://www.liv.ac.uk/researchethics/apply,for,research,ethics/.

3) Applications from Student investigators: the Committee will require proof that your Supervisor has approved the application to be submitted. Please attach this to your email. Your supervisor must be copied in on all correspondence relating to your application.

4) This form must be completed by following the guidance notes, accessible at www.liv.ac.uk/researchethics. Please complete every section, using N/A if appropriate. Incomplete forms will be returned to the applicant.

5) For studies involving overseas sites, please ensure you have researched any local approvals that might be required. Wherever possible this should include local research ethics approval. In the absence of a research ethics approval body, other relevant local approvals should be obtained, e.g. authorisation from a site, letter from a local organisation or group etc.

6) This form does not constitute insurance approval which must be sought separately. Please contact the **University's Insurance and Risk Manager** if your project involves overseas sites, vulnerable groups or is a clinical trial.

7) Staff investigators: You are encouraged to discuss your proposal with your Head of Department prior to submitting for research ethics approval.

RESEARCH MUST NOT BEGIN UNTIL ETHICAL APPROVAL HAS BEEN OBTAINED

FAILURE TO SEEK RESEARCH ETHICS APPROVAL IS TAKEN EXTREMELY SERIOUSLY BY THE INSTITUTION.

BEFORE COMPLETING YOUR APPLICATION PLEASE CONFIRM WHAT APPROVAL YOU ARE SEEKING
(Please check with "x")**:**

a) Expedited review of an individual research project X

b) Full committee review of an individual research project .....

c) Committee review generic* approval .....

*to cover a cohort of projects using similar methodologies and in line with Policy on Generic Approvals which can be found at www.liv.ac.uk/researchethics . Boundaries of the research must be defined clearly. Approval may be granted for up to 3 years and will be subject to annual review.

Declaration of the:

Principal Investigator _____ OR Supervisor and Student Investigator ___X__

(please check with a “x”)

- The information in this form is accurate to the best of my knowledge and belief, and I take full responsibility for it.
- I have read and understand the University’s Policy on Research Ethics
- I undertake to abide by the ethical principles underlying the Declaration of Helsinki and the University’s good practice guidelines on the proper conduct of research, together with the codes of practice laid down by any relevant professional or learned society.
- If the research is approved, I undertake to adhere to the study plan, the terms of the full application of which the REC has given a favourable opinion, and any conditions set out by the REC in giving its favourable opinion.
- I undertake to seek an ethical opinion from the REC before implementing substantial amendments to the study plan or to the terms of the full application of which the REC has given a favourable opinion.
- I understand that I am responsible for monitoring the research at all times.
- If there are any serious adverse events, I understand that I am responsible for immediately stopping the research and alerting the Research Ethics Committee within 24 hours of the occurrence, via ethics@liv.ac.uk.
- I am aware of my responsibility to be up to date and comply with the requirements of the law and relevant guidelines relating to security and confidentiality of personal data.
- I understand that research records/data may be subject to inspection for audit purposes if required in future.
- I understand that personal data about me as a researcher in this application will be held by the University and that this will be managed according to the principles established in the Data Protection Act.
- I understand that the information contained in this application, any supporting documentation and all correspondence with the Research Ethics Committee relating to the application, will be subject to the provisions of the Freedom of Information Acts. The information may be disclosed in response to requests made under the Acts except where statutory exemptions apply.
- I understand that all conditions apply to any co-applicants and researchers involved in the study, and that it is my responsibility to ensure that they abide by them.

- **For Supervisors**: I understand my responsibilities as supervisor, and will ensure, to the best of my abilities, that the student investigator abides by the University's Policy on Research Ethics at all times.

- **For the Student Investigator**: I understand my responsibilities to work within a set of safety, ethical and other guidelines as agreed in advance with my supervisor and understand that I must comply with the University's regulations and any other applicable code of ethics at all times.

Signature of Principal Investigator or Supe 

Date: (24/01/2016)

Print Name: Dr Francis O'Neill

Signature of Student Investigator: 

Date: (24/01/2016)

Print Name: Miss Sanaa aljamani

SECTION A - IDENTIFYING INFORMATION

A1) Title of the research *(PLEASE INCLUDE A SHORT LAY TITLE IN BRACKETS).*

Mapping of trigeminal nerve branches by using a non-invasive dental nerve stimulator

A2) PRINCIPAL INVESTIGATOR / *(PLEASE DELETE AS APPROPRIATE)*

Title:	Dr	**Staff number:**	444073
Forename/Initials:	Francis Eamon	**Surname:**	O'Neill
Post:	Senior Lecturer	**Department:**	Dentistry
Telephone:	07855481673	**E-mail:**	foneill@liv.ac.uk

A3) Student Investigator(s)

Title and Name	**Post / Current programme (if student investigator)**	**Department/ School/Institution if not UoL**	**Phone**	**Email**
Dr Sana'A Aljam'Ani	DDSc student	Dentistry	07904457482	sanaa@liv.ac.uk

A4) Co-Applicants

Title and Name	**Post / Current programme (if student investigator)**	**Department/ School/Institution if not UoL**	**Phone**	**Email**

SECTION B - PROJECT DETAILS

B1) Proposed study dates and duration (RESEARCH **MUST NOT BEGIN** UNTIL ETHICAL APPROVAL HAS BEEN OBTAINED)

Please complete as appropriate:
EITHER

a) **Starting as soon as ethical approval has been obtained**

YES *(PLEASE DELETE AS APPLICABLE)*

Approximate end date:	November 2018

OR

b) Approximate dates:

Start date:		**End date**:	

B2) Give a FULL LAY SUMMARY of the purpose, design and methodology of the planned research. *N.B. Please use as little jargon or technical language as possible. Where jargon / technical language is unavoidable, please ensure you provide a lay explanation. Please define any acronyms. The summary must be understood by persons outside of the subject area including members of the general public*

A potentially devastating complication of wisdom tooth surgery is damage to the nerve that supplies sensation and taste to the tongue and surrounding tissues. This nerve is hidden under the gum and cannot be seen by normal inspection or on routine dental x-rays. Very rarely the nerve can be seen during surgery but often a problem is only noticed after the damage has been done. This damage can result in a permanent numbness of the tongue on that side or extremely unpleasant feelings of pins and needles, pain and swelling. There are no good treatments for this damage and these symptoms remain even after surgical repair and multiple medications.

This experimental study aims to test if the position of that nerve can be located before any surgery is done so as to better avoid damaging it.

We will do this by passing a small electrical stimulus (using an already available proprietary dental nerve stimulator which is fully approved and commonly used in clinical practice. This is sterilisable and will be autoclaved between patients) over the gum until a tingling can be felt in the tongue. We will do this at several points until a line can be traced to indicate the position of the nerve. It's important to indicate that this small electrical tool is safe and has been tested previously on volunteers and showed no harm.
We intend to recruit fifty healthy participants overall. Ten will be recruited initially and if the technique is successful then we will recruit forty more. The study will require a one hour appointment during which locating the nerve and taking photographs under patients consent will be taken.
It is expected that this technique will be able to detect whether the nerve is in a vulnerable position at or above the level of the bone which puts it at risk of direct damage from instruments or drills.
In a small number of these subjects the position on the nerve will be confirmed on a magnetic reasonance imaging (MRI) scan. These scans are expensive, and therefore, not routinely used clinically but the technique has been used in the past to locate the distance of the nerve from the lower wisdom tooth for research purposes. This information would tell us how accurate our collected data from measurements made with the stimulator are. All safety protocols regarding MRI scanning will be adhered to including a checklist of exclusion criteria. Each scan will be checked by a consultant radiologist.

B3) List any research assistants, sub-contractors or other staff not named above who will be involved in the research and detail their involvement.

Professor Graham Kemp and Valerie Adams. Will perform Magnetic Reasonance Imaging at University of Liverpool Magnetic Resonance and Image Analysis Research Centre (MARIARC). This includes check listing, scanning and image preparation.

B4) List below all research sites, and their Lead Investigators, to be included in this study.

Research Site	Individual Responsible	Position and contact details
Room G02 University clinical examination room, research wing, Liverpool University Dental Hospital	Dr Francis O'Neill	Senior Lecturer/Hon Consultant in Oral Surgery. Liverpool University Dental Hospital Pembroke Place L3 5PS
University of Liverpool Magnetic Resonance and Image Analysis Research Centre (MARIARC)	Professor Graham Kemp	Director, Magnetic Resonance and Image Analysis Research Centre (MARIARC), University of Liverpool, Pembroke Place, Liverpool, L69 3GE UK

B5) Are the results of the study to be disseminated in the public domain?

YES

- ***If not, why not?***

B6) Give details of the funding of the research, including funding organisation(s), amount applied for or secured, duration, and University of Liverpool reference

Funding Body	Amount	Duration	UoL Reference
Bench fees			

B7) Give details of any interests, commercial or otherwise, you or your co-applicants have in the funding body.

Not applicable

SECTION C - EXPEDITED REVIEW

C1)

a) Will the study involve recruitment of participants outside the UK? *For studies involving overseas sites, please ensure you have researched any local approvals that might be required. Wherever possible this should include local research ethics approval. In the absence of a research ethics approval body, other relevant local approvals should be obtained, e.g. authorisation from a site, letter from a local organisation or group etc.*	No
b) Does the study involve participants who are particularly vulnerable or unable to give informed consent? *(e.g. children, people with learning or communication disabilities, people in custody, people engaged in illegal activities such as drug-taking, your own students in an educational capacity) (Note: this does not include secondary data authorised for release by the data collector for research purposes.)*	No
c) Will the study require obtaining consent from a "research participant advocate" (for definition see guidance notes) in lieu of participants who are unable to give informed consent? *(e.g. for research involving children or, people with learning or communication disabilities)*	No
d) Will it be necessary for participants, whose consent to participate in the study will be required, to take part without their knowledge at the time? *(e.g. covert observation using photography or video recording)*	No
e) Does the study involve deliberately misleading the participants?	No
f) Will the study require discussion of sensitive topics that may cause distress or embarrassment to the participant or potential risk of disclosure to the researcher of criminal activity or child protection issues? *(e.g. sexual activity, criminal activity)*	No
g) Are drugs, placebos or other substances (e.g. food substances, vitamins) to be administered to the study participants or will the study involve invasive, intrusive or potentially harmful procedures of any kind?	No
h) Will samples (e.g. blood, DNA, tissue) be obtained from participants?	No
i) Is pain or more than mild discomfort likely to result from the study?	No

j) Could the study induce psychological stress or anxiety or cause harm or negative consequences beyond the risks encountered in normal life?	No
k) Will the study involve prolonged or repetitive testing?	No
l) Will financial inducements (other than reasonable expenses and compensation for time) be offered to participants?	No

C2)

a) Will the study seek written, informed consent?	Yes
b) Will participants be informed that their participation is voluntary?	Yes
c) Will participants be informed that they are free to withdraw at any time?	Yes
d) Will participants be informed of aspects relevant to their continued participation in the study?	Yes
e) Will participants' data remain confidential?	Yes
f) Will participants be debriefed?	Yes

If you have answered 'no' to all items in SECTION C1 and 'yes' to all questions in SECTION C2 the application will be processed through expedited review.

If you have answered "Yes" to one or more questions in Section C1, or "No" to one or more questions in Section C2, but wish to apply for expedited review, please make the case below.

C3) Case for Expedited Review – *To be used if asking for expedited review despite answering YES to questions in C1 or NO to answers in C2.*

N/A

SECTION D - PARTICIPANT DETAILS

D1) How many participants will be recruited?

50

D2) How was the number of participants decided upon?

From anatomical studies it is estimated that the lingual nerve may be in a surgically unfavourable position in approximately 10% of the population. To be reasonably certain that a similar proportion of subjects can be identified and accurately detected in a random population sample, 50 participants will be recruited to detect 5 participants with unfavourable positioning of the lingual nerve. The tests can be completed in a short timeframe and are not onerous on the part of the participant or researcher. Therefore it is realistic that this number of subjects can be recruited and tested within the timeframe given.

D3)

a) Describe how potential participants in the study will be identified, approached and recruited.

Recruitment will be open to anyone who sees the advertisement media and who meets the inclusion/exclusion criteria. As this media will be placed in appropriate media boards on university campus it is envisaged that recruits will be mostly from staff, students, family and friends, and other university visitors. Recruits will be healthy volunteers. Recruitment will NOT include NHS patients. 1. Posters Designated advertisement posters will be attached on designated notice boards with high visibility positions on university property. 2. E-mail An email with a short text explaining the study, and containing contact information details, may be sent to key secretarial staff for distribution to

staff or students of the university via their university email account. One follow-up email only will be sent as a reminder.

3. Oral presentation

Providing there is prior agreement, it may be possible at the end of the some key lectures to invite audience members to listen to a brief description of the study and to give contact details to discuss any further enquiries or questions.

b) Inclusion criteria:

1. Subjects will be at least 18 years old
2. Wisdom teeth still in situ/ not known if extracted

c) Exclusion criteria:

1. Oral mucosal conditions in the anatomical area of testing
2. Untreated or uncontrolled medical conditions

d) Are any specific groups to be excluded from this study? If so please list them and explain why:

No specific groups will be excluded except those in vulnerable groups identified in Section 3 of this application.

e) Give details for cases and controls separately if appropriate:

N/A

f) Give details of any advertisements:

1) Poster: please see attached poster. Designated advertisement posters will be attached on designated notice boards with high visibility positions on university property.

2) E-mail: An email with a short text explaining the study, and containing contact information details and an attached poster, may be sent to key secretarial staff for distribution to staff or students of the university via their university email account. One follow-up email only will be sent as a reminder.

3) Oral presentation: **if agreed, after specified lectures or meetings, we can verbally draw attention to the study by giving the audience a short description of the project and contact details to discuss any further enquiries or questions.**

D4)

a) State the numbers of participants from any of the following vulnerable groups and justify their inclusion

Children under 16 years of age:	0
Adults with learning disabilities:	0
Adults with dementia:	0
Prisoners:	0
Young Offenders:	0
Adults who are unable to consent for themselves:	0
Those who could be considered to have a particularly dependent relationship with the investigator, e.g. those in care homes, students of the PI or Co-applicants:	0
Other vulnerable groups (please list):	0

b) State the numbers of healthy volunteer participants:

Healthy Volunteers	**50**

D5)

a) Describe the arrangements for gaining informed consent from the research participants.

Consent will be gained following a full explanation of the study and the nature of the experimental procedure. This will be given in a patient information sheet and also verbally. The potential participant will have an opportunity to have any questions about this answered and also will be given the opportunity to have a further period of time to consider the information. Only when the potential participant feels comfortable they have understood and weighed the information will they sign the consent form. All participants will be informed that consent, and therefore participation, is entirely voluntary and can be withdrawn at any point without the need to give any reason.

b) If participants are to be recruited from any of the potentially vulnerable groups listed above, give details of extra steps taken to assure their protection, including arrangements to obtain consent from a legal, political or other appropriate representative in addition to the consent of the participant *(e.g. HM Prison Service for research with young offenders, Head Teachers for research with children etc.).*

N/A

c) If participants might not adequately understand verbal explanations or written information given in English, describe the arrangements for those participants *(e.g. translation, use of interpreters etc.)*

It is not expected that this situation will be encountered given that the healthy volunteer pool will most likely be from the student population who by necessity will speak English to a fluent level. If the experiment shows utility then this naturally would be rolled out to include all subjects it may benefit. In this instance funding will be applied for to cover costs of translational services if they are required.

d) Where informed consent is not to be obtained (including the deception of participants) please explain why.

N/A

D6) What is the potential for benefit to research participants, if any?

In a certain proportion of participants where an abnormal anatomical position of the nerve branch is demonstrated this may potentially benefit any of those participants that may subsequently go on to have surgery in that area as prior knowledge of this anomaly may inform planning and reduce risk of nerve damage.

D7) State any fees, reimbursements for time and inconvenience, or other forms of compensation that individual research participants may receive. Include direct payments, reimbursement of expenses or any other benefits of taking part in the research?

A small reimbursement of no more than £10 per hour to compensate for the inconvenience of travel and experimental time will be offered.

SECTION E - RISKS AND THEIR MANAGEMENT

***NOTE**: Completing section E fulfils the requirement for risk assessment, provided that this section is reviewed if circumstances change, or new information makes it necessary.*

A copy of this form should be given to your departmental safety coordinator to enable monitoring of risk assessments. The findings of the risk assessment, especially the precautions required, must be communicated in a user-friendly way to all those doing this work.

E1) Describe in detail the potential physical or psychological adverse effects, risks or hazards (minimal, moderate, high or severe) of involvement in the research for research participants.

This technology has been used for several decades in the clinical setting and no adverse events have been reported. The procedure is simple, safe, temporary, reversible and fully in the control of the subject. Therefore it is not expected that any physical or psychological adverse effects will be encountered.
There is a theoretical risk of a temporary electric shock like experience if the stimulator is applied on full power. This risk will be minimised by ensuring that any stimulation is started at the lowest setting possible and only increased slowly until perceived by the subject. Each incremental increase in stimulation setting will only be performed if agreed by the subject.

E2) Explain how the potential benefits of the research outweigh any risks to the participants.

The potential benefit of the research is that this technique may be useful in identification of aberrant nerve anatomy. This information may in turn be used to reduce the risk of permanent nerve damage during surgery. Permanent nerve damage often results in distressing symptoms such as numbness, tingling (pin and needles), pain or a sensation of swelling in the distribution of the affected nerve. As any risk to the subject during the experiment is temporary, brief and fully reversible it is clear that the benefit of the research (at least on the population as a whole) greatly outweighs the risk.

E3) Describe in detail the potential adverse effects, risks or hazards (minimal, moderate, high or severe) arising from this research to the researchers or anyone else.

The only potential risk to subjects are from cross-infection through saliva contamination. All probes will be sterilised between uses using standard protocols and investigators will apply standard cross-infection protocols during the procedures.

E4) What precautions will be in place to minimise the risks identified in E1 and E3?

As described, the experimental protocol will specifically state starting the experimental stimulus at the lowest possible setting and include the slow, incremental increase in stimulation strength at the discretion of the subject and within comfortable limits. Subjects will be clearly instructed how to break the circuit to cease stimulation at will (this will involve merely letting go of a conduction wire) if any discomfort is felt. Standard cross-infection protocols will be applied during all procedures.

E5) Will individual or group interviews/questionnaires discuss any topics or issues that might be sensitive, embarrassing or upsetting, or is it possible that criminal or other disclosures requiring action could take place during the study (e.g. during interviews/group discussions, or use of screening tests for drugs)**?**

NO *(PLEASE DELETE AS APPLICABLE)*

- ***If Yes, give details of procedures in place to deal with these issues.***

N/A

E6) Describe the measures in place in the event of any unexpected outcomes or adverse events to participants arising from their involvement in the project

The lead researcher is a dual medically and dentally qualified clinician with a specific specialist interest in facial pain and trigeminal neuroscience. With regards to MRI scanning, images will be checked by a consultant radiologist and in the unlikely event, any incidental findings will be managed by the clinical researchers involved, whose responsibility it will be to

communicate any significant abnormality discovered to the subject's GP, if necessary obtaining an expert opinion from suitably qualified specialist colleagues.

E7) Explain how the conduct of the project will be monitored to ensure that it conforms with the study plan and relevant University policies and guidance.

The study will be conducted under the supervision of the principle investigator in compliance with university policies and research governance guidance.

SECTION F - DATA ACCESS AND STORAGE

F1) Where the research involves any of the following activities at any stage (including identification of potential research participants), state what measures have been put in place to ensure confidentiality of personal data *(e.g. encryption or other anonymisation procedures will be used).*

**PLEASE NOTE THAT UNLESS THERE ARE EXCEPTIONAL CIRCUMSTANCES, ALL DATA MUST BE HELD SECURELY ON THE "M" DRIVE AND IN LINE WITH UNIVERSITY POLICY. VISIT THE CSD WEBPAGES FOR FURTHER INFORMATION*

Electronic transfer of data by magnetic or optical media, e-mail or computer networks	All data will be stored the university "M" drive and on a university issued Laptop computer with access protected by password. All files will also be password protected. Any necessity for transfer between devices other than through the university Ethernet connection to the server will be via an encrypted password protected USD drive.
Sharing of data with other organisations	N/A
Exporting data outside the European Union	N/A
Use of personal addresses, postcodes, faxes, e-mails or telephone numbers	N/A
Publication of direct quotations from respondents	N/A
Publication of data that might allow identification of individuals	N/A
Use of audio/visual recording devices	Photographs of mapped nerves will be taken as part of the record. These will be intra-oral photographs of a small area of mucosa only and therefore will not include any patient identifiable information. Anonymised but numbered photographs will be stored on the university server.

	Photos will be deleted from the internal camera data storage card.
Storage of personal data on any of the following:	
Manual files	Subjects who are at benefit from the study (i.e. are shown to have aberrant nerve anatomy) will be given a copy of their own photograph documenting the nerve anatomy to be kept by the subject in case of the need arises for surgery in that area. This can then be used at the discretion of the subject if necessary.
Home or other personal computers	*DATA MUST ONLY BE STORED ON THE UNIVERSITY'S SECURE SERVER, YOU CAN GAIN REMOTE ACCESS TO THE SECURE SERVER VIA THE UNIVERSITY'S APPS ANYWHERE APPLICATION.**
University computers	*DATA MUST ONLY BE STORED ON THE UNIVERSITY'S SECURE SERVER, YOU CAN GAIN REMOTE ACCESS TO THE SECURE SERVER VIA THE UNIVERSITY'S APPS ANYWHERE APPLICATION.**
Private company computers	*DATA MUST ONLY BE STORED ON THE UNIVERSITY'S SECURE SERVER, YOU CAN GAIN REMOTE ACCESS TO THE SECURE SERVER VIA THE UNIVERSITY'S APPS ANYWHERE APPLICATION.**
Laptop computers	*DATA MUST ONLY BE STORED ON THE UNIVERSITY'S SECURE SERVER, YOU CAN GAIN REMOTE ACCESS TO THE SECURE SERVER VIA THE UNIVERSITY'S APPS ANYWHERE APPLICATION.**

F2) Who will have control of and act as the PRIMARY custodian for the data generated by the study?

PRINCIPAL INVESTIGATOR
(PLEASE DELETE AS APPLICABLE)

F3) Who will have access to the data generated by the study?

Principle investigator and student investigator

F4) For how long will data from the study be stored?

Data will be stored for no more than 5 years. All data will be stored the secure university "M" drive and on a secure university issued Laptop computer with access protected by password. All files will also be password protected. Any necessity for transfer between devices other than through the university Ethernet connection to the server will be via an encrypted password protected USD drive.

<u>SECTION G – PEER REVIEW AND TRAINING</u>

G1) a) Has the project undergone peer review?

YES *(PLEASE DELETE AS APPLICABLE)*

b) If yes, by whom was this carried out? *(please enclose evidence if available)*

As part of the university DDSc submission process this application has been peer reviewed by Dr Sarah Lyon and Professor Callum Youngson at proposal stage. Recommended changes have been made since this stage. A copy of Professor Youngsons making of the proposal has been included with the ethics submission.

G2) a) What date was your most recent training in research ethics?

Date:	Certificate Oct 2015

b) Please provide details of the training provider and course:

Training provider:	NIHR
Course title:	Good Clinical Practice

SECTION H - CHECKLIST OF ENCLOSURES

PLEASE ADD "YES"
WHERE APPROPRIATE

Study Plan / Protocol	yes
Recruitment advertisement	yes
Participant information sheet	yes
Participant Consent form	yes
Research Participant Advocate Consent form	
Evidence of external approvals	
Questionnaires on sensitive topics	
Interview schedule	
Debriefing material	
Other (please specify)	
Evidence of peer review (If G1 = Yes)	yes

UNIVERSITY OF LIVERPOOL

Research Study Risk Assessment

Scoring Matrix

IMPACT	LIKELIHOOD: Remote	Unlikely	Possible	Likely	Certain
Low	1	2	3	4	5
Moderate	2	4	6	8	10
Significant	3	6	9	12	15
Severe	4	8	12	16	20
Catastrophic	5	10	15	20	25

25

RISK	Very Low	Low	Moderate	High	Very High

To rate a risk:

1. Grade the impact of the worse case scenario
2. Multiply this impact [1-5] by the likelihood [1-5], to get your rating.

Action and time scales
Immediate action must be taken to manage the risk. Control measures should be put in place which will have the effect of reducing the impact of an event or the likelihood of an event occurring. A number of control measures may be required.
Significant resources may have to be allocated to reduce the risk. Where the risk involves work in progress urgent action should be taken.

Version 1 (12/05/2016)

Action and time scales
Immediate action must be taken to manage the risk. Control measures should be put in place which will have the effect of reducing the impact of an event or the likelihood of an event occurring. A number of control measures may be required.
Significant resources may have to be allocated to reduce the risk. Where the risk involves work in progress urgent action should be taken.
Efforts should be made to reduce the risk, but the costs of prevention should be carefully measured and weighed against the impact of the event. Establish more precisely the likelihood of harm as a basis for determining the need for improved control measures.
On or below this level a risk is acceptable. Existing controls should be monitored and adjusted. No further action or additional costs are required. Consideration may be given to a more cost-effective solution or improvement that imposes no additional cost burden.
Acceptable risk. No further action or additional controls are required. Risks at this level should be monitored, and reassessed at appropriate intervals.

UNIVERSITY OF LIVERPOOL

Research Study Risk Assessment

Scoring Matrix

IMPACT	LIKELIHOOD: Remote	Unlikely	Possible	Likely	Certain
Low	1	2	3	4	5
Moderate	2	4	6	8	10
Significant	3	6	9	12	15
Severe	4	8	12	16	20
Catastrophic	5	10	15	20	25

RISK		Very Low		Low		Moderate		High		Very High

To rate a risk:

1. Grade the impact of the worse case scenario
2. Multiply this impact [1-5] by the likelihood [1-5], to get your rating

Action and time scales
Immediate action must be taken to manage the risk. Control measures should be put in place which will have the effect of reducing the impact of an event or the likelihood of an event occurring. A number of control measures may be required.
Significant resources may have to be allocated to reduce the risk. Where the risk involves work in progress urgent action should be taken.

Version 1 (12/05/2016)

Participant Hazards (Rights and Safety)							
Generic Hazard	**Impact (I)** 1 - 5	**Likelihood (L)** 1 - 5	**Risk (R)** IxL	**Specific Hazard**	**Management Strategies**	**Central Monitoring**	**Monitoring At Sites**
Side effects	1 1	1 3	1 3	Electric pulp tester device 1. Cross infection 2. Pain on stimulation	1. Standard cross infection protocols followed. Sterilisation by autoclave. 2. Stimulation monitored during procedure every time stimulation setting changed. Advice included in information leaflets.	CI	One site only
Improper consent	1	2	2	Absent, uninformed or pressurised consent	Consent only to be done by appropriately trained (GCP certified) staff.	CI	One site only
MRI related events	3 3	1 1	3 3	1. Adverse event during MRI scan 2. Incidental finding	Full assessment checklist to be completed prior to scan. Images to be reported by radiologist and incidental finding protocol followed	CI	Director MARIARC
			12				

Study Hazards (Completion and Reliability)							
Generic Hazard	**Impact (I)** 1 - 5	**Likelihood (L)** 1 - 5	**Risk (R)** IxL	**Specific Hazard**	**Management Strategies**	**Central Monitoring**	**Monitoring At Sites**
Failure of participant recruitment	1	1	1	Attrition of participants	Minimal risk due to short nature of study and only 1-2 visits required. Will recruit until number reached.	CI	One site only
Staff competence	3	1	3	Inappropriate participant advice	Staff/student training.	CI	One site only
Study results	3	1	3	Incomplete or improperly completed data collection forms.	Student training, data collection only by GCP trained member.	CI	One site only
			7				

Study Hazards (Completion and Reliability)							
Generic Hazard	**Impact (I)** 1 - 5	**Likelihood (L)** 1 - 5	**Risk (R)** IxL	**Specific Hazard**	**Management Strategies**	**Central Monitoring**	**Monitoring At Sites**
Failure of participant recruitment	1	1	1	Attrition of participants	Minimal risk due to short nature of study and only 1-2 visits required. Will recruit until number reached.	CI	One site only
Staff competence	3	1	3	Inappropriate participant advice	Staff/student training.	CI	One site only
Study results	3	1	3	Incomplete or improperly completed data collection forms.	Student training, data collection only by GCP trained member.	CI	One site only
			7				

Appendix 3 Acceptance letter from the ethics committee that was gained prior to the start of the study.

UNIVERSITY OF LIVERPOOL

Dr Mantalena Sotiriadou
Research Ethics and Integrity Officer
University of Liverpool
Correspondence to;
Research Support Office
University of Liverpool
Waterhouse Building
2nd Floor, Block D
3 Brownlow Street
Liverpool
L69 3GL

Email: ethics@liverpool.ac.uk
Telephone: 0151 795 8355

7 June, 2016

Dear Dr O'Neill,

Re: Ethical Approval

I am pleased to inform you that your study has been approved. Details and conditions of the approval can be found below:

Ethics reference number	RETH001033
Review type:	Full committee review
Committee name:	Research Ethics Sub-committee for Physical Interventions
Principal Investigator:	Dr Francis Eamon O'Neill
Student Investigators:	Dr Sana'A Aljam'Ani
Department:	Dentistry
Title:	Mapping of trigeminal nerve branches by using a non-invasive dental nerve stimulator
First reviewer:	Dr David Taylor
Date of approval:	07/06/16
Approximate end date:	30/11/18

The application was APPROVED subject to the following conditions:

Conditions

- All serious adverse events must be reported to the Subcommittee within 24 hours of their occurrence, via the Research Integrity and Governance Officer (ethics@liverpool.ac.uk).
- This approval applies for the duration of the research. If it is proposed to extend the duration of the study as specified in the application form, the Subcommittee should be notified, via the Research Integrity and Governance Officer (ethics@liverpool.ac.uk).
- If it is proposed to make an amendment to the research, you should notify the Committee by following the Notice of Amendment procedure. If the named PI / Supervisor leaves the employment of the University during the course of this approval, the approval will lapse. Therefore please contact the Research Integrity and Governance Officer at ethics@liverpool.ac.uk in order to notify them of a change in PI / Supervisor.

Yours sincerely,

Dr Mantalena Sotiriadou
Research Ethics and Integrity Officer

Appendix 4 Designated poster for recruiting participants in the study.

participants are needed!!

UNIVERSITY OF LIVERPOOL

SCHOOL OF DENTISTRY

Help us prevent nerve injury and make surgery safer

If you are healthy, have no oral ulceration, over 18 years old, and have not had your lower wisdom teeth removed
You can help us develop a simple pain-free test
NO SURGERY NEEDED!!

one hour appointment

Amazon voucher £10/hr

For further information about the study and participation please contact Us:

Dr Francis O'Neill (foneill@liv.ac.uk)
Miss Sanaa aljamani (sanaa@liv.ac.uk)

Appendix 5 Copy of designated email as part of recruitment process.

Dear Colleagues:

We are looking for healthy participants to take place in our dental research project. If you are above 18 years of age, and still have your wisdom teeth, you can be one of our participants. The study will take place on Thursdays and Fridays AM with considering Lunch time (1-2 pm) in week days for participants who are busy during working hours.

If you are interested in participation please contact Miss Sanaa Aljam'Ani (sanaa@liv.ac.uk) with giving the most suitable time option amongst the above mentioned time slots so the arrangement will be made based on that. Your help would be much appreciated. For further details please contact us

Dr Francis O'Neill (foneill@liv.ac.uk)

Miss Sanaa Aljam'Ani (sanaa@liv.ac.uk

Appendix 6 Participant's information leaflet for the first and second phases of the study.

Participant information leaflet

Study: Clinical mapping of trigeminal nerve branches

You are being invited to participate in a research study. Before you decide whether to participate, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and feel free to ask us if you would like more information or if there is anything that you do not understand. Please also feel free to discuss this with your friends, relatives and GP if you wish. We would like to stress that you do not have to accept this invitation and should only agree to take part if you want to.

What is the study about?

This study aims to map the position of several nerves that are hidden under the gum in the mouth. These nerves cannot be seen on x-rays and their location is often guessed during surgery. However, if they are damaged during surgery they can leave permanent changes in sensation including numbness of the tongue or lip. If the position of the nerve is known then it may be easier to avoid during the procedure.

What is done in the study?

In this study a researcher a looks for the position of the nerve lying under the gum by sweeping the area with a small electrical stimulus probe (pic 1). When the stimulus probe is on the gum it feels like a mild tingling just in under the probe tip. But when the probe is over the nerve it feels like a tingling sensation in the tongue or lip depending on the nerve being studied.

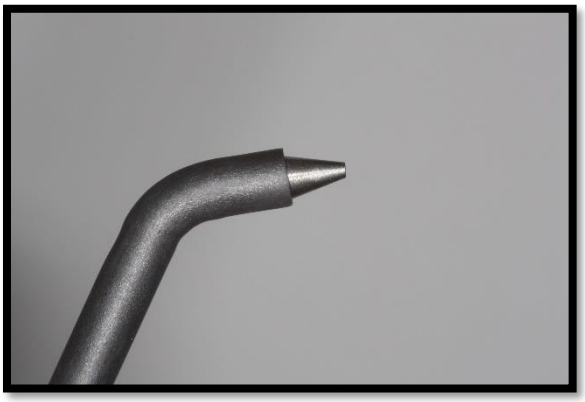
When the nerve is found like this the researcher will mark the spot with a special pen and repeat the procedure until the path of the nerve can be traced between 3 or 4 dots. The researcher will then take a photograph of the line of the nerve. The photograph will only be a picture of a small area of the mouth (pics 2-4) and will not contain information about your identity. This procedure will then be repeated on the other side of the mouth.

What can I expect?

The whole procedure will take approximately 20-30 minutes. You will be invited to attend the Liverpool University Dental Hospital where the researcher (Dr O'Neill) will carry out the mapping. This will involve drying the area of gum to be studied, demonstrating how the probe is used and then beginning with the stimulus set to the very lowest level and slowly increasing it until you can just notice a mild tingling. The probe will then be moved in sweeping movements until you feel a sensation of tingling in the tongue or lip. As soon as this is felt you can stop the stimulation and the exact spot will be marked

with a pen special marker pen. This procedure is repeated until there are several marker dots with which to draw the line of the nerve. This line will then be photographed and the marker ink can be removed. This will be the end of the appointment.

There may be a residual feeling of tingling in the lip or tongue for a short time after the session, this is normal and will quickly go away.



Picture 1

The probe tip is blunt and does not harm the gum

Pictures 2-4

The probe is placed gently against the gums and slowly swept forward in several straight lines.

What are the benefits to me?

If it is discovered that the nerve we are studying happens to be in an area where surgery may be needed, (for

example wisdom tooth removal) then you can have a copy of the photograph to give to whoever is going to do the surgery for them to consider before the procedure. This may help them plan the surgery.

What are the risks to me?

There are no direct disadvantages. There may be a residual feeling of tingling in the lip or tongue for a short time after the session, this is normal and will quickly go away.

Who is able to enter the study?

Anyone who is aged between 18-70 years old with at least one wisdom tooth can enter the study unless you have one of the following specific conditions:

1. Abnormal sensation or numbness in the tongue or lip
2. Very strong gagging reflex
3. Ulceration or similar condition of the gum in the area being studied
4. Heart condition that requires a pacemaker

What happens if I want to stop before you have competed the session?

Consent is always completely voluntary and up to you. You can stop at anytime without the need to give a reason.

What else is included in the study?

A small number of participants were the lingual nerve has been successfully mapped will be invited to go for a scan of their face to visualise and corroborate the position of the lingual nerve with the test results. This will be done in

an MRI scanner in a research centre located across from the dental hospital. If you are invited this would require one further appointment for the scan which will take approximately one hour. A separate checklist will be completed prior to this to ensure there are no reasons why you should not have the scan. A separate information leaflet is available with regards to this part of the study.

Expenses

Expenses will be reimbursed at the rate of £10 per hour/session in the form of amazon online retailer vouchers

What will happen to results of the study?

Results will be submitted for publication in a peer review journal to inform members of the dental specialty of the findings. They will also be included in a doctoral thesis which will be submitted to the University of Liverpool.

Contacts

Dr Francis O'Neill	Sanaa Aljamani
University of Liverpool	University of Liverpool
Liverpool University Dental Hospital	Liverpool Dental Hospital
Pembroke Place	Pembroke Place
Liverpool	Liverpool
L3 5PS	L3 5PS
Tel 0151 706 5245	Tel 5240
Email: foneill@liv.ac.uk	sanaa@liverpool.ac.uk

Appendix 7 Consent form for participants prior to study conduction.

CONSENT FORM

Title of Project: Mapping of intra-oral nerve fibre branches

Investigators:

Dr. Francis O'Neill, Ph.D., MBChB, FDS (OS) RCPS

Senior Lecturer/Honorary Consultant in Oral Surgery

Miss Sanaa Aljamani Postgraduate DDSc student in Endodontology

1. I confirm that I have read, and I understand the information sheet dated 12/05/2016 (version 2) for the above study and have had the opportunity to ask questions.
2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason.
3. I agree to take part in the above study.
4. I understand that the pictures taken of the inside of my mouth contain no personally identifiable information and that they may be used for publication or future studies. Under the Data Protection Act, I can at any time ask for access to the information I provide, and I can also request the destruction of that information if I wish.
5. I understand that some of my data may be retained and may be used for further research purposes

…… …… ……..

Name of Participant Signature Date

…… …… ……..

Name of person Signature Date

taking consent

Appendix 8 Data collection form of the first, second and clinical reliability study:

Participant name

number:

Gender:

Age:

Consent and PIL:

	Right side	Left side
Eruption:		
Point A (Vertical Retromolar pad)		
Point B (Vertical middle of 3rd molar)		
Point C (Vertical distal of the 7)		
A-C horizontal		
EPT max reading		

<u>Adverse, Unpleasant events (out of 10):</u>

Pressure of EPT on keratinized gingivae:

Pressure of EPT on non-keratinized gingivae:

Electrical stimulus:

Lasting sensation:

Unpleasant sensation:

Would you consider this test prior XLA of lower 8 in the future?

Additional Notes:

Appendix 9 : MRI participant information leaflet.

Participant information leaflet
(Part 3) MRI scan

Study: Clinical mapping of trigeminal nerve branches

What is the study about?

This study is an additional part to the study on the mapping of the trigeminal nerve you have already taken part in. As we have been able to map your nerves, we would like to invite you to have a small scan of this mapped area to confirm the location of these nerves.

What is done in the study?

This part of the study requires you to have an MRI scan of the head so we can look at the area we have been mapping. The scan will be completed in a single appointment and should take no longer than 30-40 minutes. It will be done at the MARIARC centre across the road form Liverpool University Dental Hospital.

What will happen to me if I take part?

You will be asked to fill in a safety screening form to make sure there are no reasons why you would not be suitable for magnetic resonance scanning. You will be asked to wear a gown (changing rooms are provided) and remove items which are affected by the magnetic field (e.g. hearing aids, mobile phones, keys, coins, pens, credit cards (secure lockers are provided). MR scans are noisy so you will need to wear the ear protection that will be provided. MR scans cause no pain, harm or long-term effects. Some people may experience slight feelings of claustrophobia in the scanner. If you do feel uncomfortable, you will be able to notify us immediately, and we will remove you from the scanner without delay

What are the possible disadvantages and risks of taking part?

There are no known risks in properly conducted magnetic resonance scanning. As it involves a strong magnetic field, certain standard precautions will be observed. Most importantly, we will NOT study you if you are fitted with a heart pacemaker, mini-defibrillator or a neurostimulator; if you have surgical clips in your head; if you have suffered injuries which may have left metal particles in your eye or head, or elsewhere in your body; or if you have an artificial heart valve. We will also ask about other kinds of surgery and metal implant which might affect your suitability. Some people find the scanner a claustrophobic or uncomfortable environment, and we will ask you about this.

Occasionally research studies using magnetic resonance imaging reveal significant unexpected abnormalities which require medical follow-up, either for further investigation or (more rarely) treatment. The scans we do are for research purposes, but we review them carefully to avoid missing such an abnormality. If you have a scan of your brain or spinal cord, we will spend a few extra minutes taking high-quality images which we will routinely have reviewed by a consultant radiologist. If any significant abnormality is found, we will send the report to your GP, who will be able to take it further with you. Please note that this is not a substitute for a 'medical' magnetic resonance scan that a doctor might order to make a diagnosis. It should therefore not be seen as a 'health check.'

Will information about me be kept confidential?

All information that is collected about you during the research will be kept strictly confidential by the researchers. Your data related to the MR study will be treated in an anonymous way. Your personal information that is collected on the safety screening form will be kept for up to 15 years, and then will be confidentially destroyed. You have a legal right to view your personal information stored with us. If you wish to view your

personal information, please write to the University Data Protection Officer, Legal, Risk & Compliance, and University of Liverpool.

Will my taking part be covered by an insurance scheme?

Participants taking part in the University of Liverpool ethically approved study will have insurance cover.

What will happen to the results of the study?

The results of the research study will be presented at research meetings and published in the scientific literature so that other researchers can also benefit from the sharing of information. The study will take at least *two years* to conduct and longer to analyse fully, but we would be happy to supply you with our final results after this time.

What will happen if I want to stop taking part?

During the study, you can withdraw at any time without explanation. Your results up to the period of withdrawal may be used if you are happy for this to be done. Otherwise, you may request that they are destroyed and no further use is made of them.

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Appendix 10 NHS MRI safety checklist that was used for screening participants in this study as part of the study protocol.

NHS number: _____

Name: _____

Address: _____

_____

Date of birth: _____

CR number: _____

Royal Cornwall Hospitals NHS
NHS Trust

MRI SAFETY CHECKLIST

Please answer the following questions		Yes / No
1	Do you have a cardiac pacemaker/defibrillator?	
2	Have you ever had a cardiac pacemaker/defibrillator? If yes, give details	
3	Have you ever had heart surgery? If yes, give details	
4	Do you have a neuro-stimulator?	
5	Have you ever had any type of electronic, mechanical, or magnetic implant? If yes, give details	
6	Have you ever had surgery to your brain? If yes, give details	
7	Do you have a programmable hydrocephalus shunt?	
8	Have you ever had surgery to your ears? If yes, give details	
9	Have you ever had surgery to your eyes? If yes, give details	
10	Have you ever had any operations involving the use of metal implants, plates, or clips? If yes, give details	

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MRI SAFETY CHECKLIST cont...

Please answer the following questions		Yes / No
11	Have you ever had any metal fragments in your eyes? If yes, give details	
12	Have you ever had any metal fragments in any other part of your body? If yes, give details	
13	Have you had any surgery in any part of your body in the past 6 weeks? If yes, give details	
14	Do you have a prosthetic limb, eye or other artificial device not already mentioned? If yes, give details	
15	Are you wearing any medication patches? (eg. Nicotine, HRT patch)	
16	Do you have any wound dressings?	
17	Have you had a previous MRI scan? If yes, when was the most recent?	
18	What is your weight? (Stones) (Kilograms)	
	WOMEN ONLY	
19	Could you be pregnant?	
20	Are you breast-feeding?	

I confirm that that the information I have provided is correct to the best of my knowledge.

Signature of Patient:	Date:
Signature of Staff Member (1):	Date:
Signature of Staff Member (2):	Date:

Please remove all loose metallic objects, including metallic body piercing, hearing aids, foil drug patches and dentures.